Remarks

The above amendments and the following remarks are believed to address the requirements set forth by the examiner in the previous Office Action and put the claims in better form. It is respectfully requested that the Examiner enter the amendments and reconsider this Application.

Upon entry of the foregoing amendments, claims 29-60 are pending in the application, with claim 29 being the independent claim. Claims 1-28 are sought to be cancelled without prejudice to or disclaimer of the subject matter therein. New claims 29-60 are sought to be added. Support for the new claims can be found in the previously presented claims 1-3 and 11-28, Figure 1a, and at paragraphs [0059] and [0117]-[0124] of the published application. These changes are believed to introduce no new matter, and their entry is respectfully requested.

Based on the above amendments and the following remarks, Applicants respectfully request that the Examiner reconsider all outstanding objections and rejections and that they be withdrawn.

Objections to the Claims

The Examiner objected to claim 16 because of informalities. Claim 16 has been cancelled. Insofar that the objection applies to the currently pending claim 47, Applicants have removed the informalities. Thus, Applicants respectfully request that this objection be reconsidered and withdrawn.

Rejections under 35 U.S.C. § 112

35 U.S.C. § 112, first paragraph (scope of enablement)

The Examiner has rejected claims 16 and 19-28 under 35 U.S.C. § 112, first paragraph. *See* Office Action, hereinafter "OA", at pages 3-9. The Examiner asserts that the specification while "being enabling for methods of preventing or treating cancer, inducing antitumor immunity, reducing tumor growth, decreasing tumor metastasis and prolonging survival period" in a rodent "does not reasonably provide enablement for a method of "preventing or treating an enormous genus of etiologically and pathologically distinct cancers in an enormous genus of mammalian subjects, including humans." OA at pages 3. Claims 16 and 19-28 have been cancelled. Insofar that the rejection applies to the currently pending claims 47-60, Applicants respectfully traverse this rejection.

The Examiner's argument

The Examiner asserts that "the substantive issue is whether or not the rodent model provides enabling support for a method of preventing or treating an enormous genus of biologically and physiologically distinct mammalian organisms" OA at page 8. The Examiner asserts, "[t]he art has recognized that many *in vitro* and animal models that are provided as evidence of success of treatment have not translated into successful treatment in humans. Eliciting anti-tumor immunity in [human] cancer patients using DNA vaccines has proved more difficult, and little evidence of anti-tumor immunity was demonstrated." OA at page 8. Applicants respectfully traverse this rejection.

The legal standard

The test of enablement is whether one reasonably skilled in the art could make or use the invention from the disclosure in the specification coupled with information known in the art

without undue experimentation. *In re Wands*, 858 F.2d 731, 737 (Fed. Cir. 1988). The captioned application has provided working examples in which a preventative or therapeutic model showed antitumor immunity against human Her-2/neu expressing murine tumor cells, Applicants assert that the captioned application is enabled for methods of preventing or treating mammalian organisms.

The Applicants' argument

The claims of the present invention are fully enabled. Claims 47-60 are presently directed to methods for preventing or treating Her-2/neu-over-expressing cancers. The references cited by the Examiner and the arguments set forth in view of those references do not cast doubt on the feasibility of the claimed invention in light of the data presented in the specification. While the Examiner argues the instant application "does not reasonably provide enablement for a method of "preventing or treating an enormous genus of etiologically and pathologically distinct cancers in an enormous genus of mammalian subjects, including humans[,]" Applicants point out that the method claims are currently drawn to "Her-2/neu-over-expressing cancers", which are *specific* etiologically and pathologically distinct cancers. As discussed below, the current application describes an animal model that reasonably correlates with preventative or therapeutic treatment in humans.

The Examiner asserts that "[w]ith respect to the method, the claim is broad for encompassing treatment methods as applied to an enormous genus of subjects, including humans[.]" OA at page 4. The Examiner is reminded that mere breadth of a claim does not make a claim not enabled or indefinite as long as the scope of the subject matter that is embraced is clear. *In re Miller*, 441 F.2d 689 (CCPA 1971). *See* MPEP 2173.04.) Additionally, it is permissible for claims to encompass inoperative embodiments. *See* MPEP 2164.08(b). The

presence of inoperative embodiments within the scope of a claim does not necessarily render a claim nonenabled. *Atlas Powder Co. v. E.I. du Pont de Nemours & Co.*, 750 F.2d 1569, 1577, (Fed. Cir. 1984) (prophetic examples do not make the disclosure nonenabling). A disclosure of a large number of operable embodiments and the identification of a single inoperative embodiment did not render a claim broader than the enabled scope because undue experimentation was not involved in determining those embodiments that were operable. *In re Angstadt*, 537 F.2d 498, 502-503, 190 USPQ 214, 218 (CCPA 1976).

Using the methods of the current invention would not require undue experimentation. The facts in *In re Gardner, Roe and Willey*, 427 F.2d 786, 798 (C.C.P.A. 1970) cited by the Examiner, differ significantly from the facts in the present application. In *In re Gardner, Roe and Willey*, the court stated that the specification did not provide "an illustration of how the invention is to be practiced on any kind of host." *Id.* The experiments testing an antidepressant drug were done in rats and "rats, not being human, do not exhibit mental depression." *Id.* at 790.

Here, the present application has provided working examples in which a truncated Her-2/neu DNA vaccine was effective at providing antitumor immunity against human Her-2/neu expressing murine tumor cells (Her-2-CT26) in mice. Mice, like humans, are susceptible to tumors. Thus, contrary to the facts in *In re Gardner, Roe and Willey,* the present specification reasonably enables the full scope of the claims without undue experimentation. The Examiner is reminded that an enabling disclosure allows for some experimentation even in large amounts as long as it is not undue. *In re Wands*, 858 F.2d 731, 737 (Fed. Cir. 1988). In addition to working examples, the specification includes guidance for administration, formulation and dosage of the plasmid constructs which a person of ordinary skill would find sufficient to make and use the

claimed invention. *See, e.g.*, Specification at [0064] - [0068]. Thus, the specification has provided a clear road map to allow the ordinary artisan in the field of immunology to practice the invention without undue experimentation.

The Examiner must provide reasons for the assertion that the disclosure is not enabled and unbelievable. *In re Bowen*, 492 F.2d 859, 862-63 (CCPA 1974). The Examiner alleges that the art of gene therapy is unpredictable citing a weblink to a Washington Post article. OA at page 8. Although unpredictability in the art may provide reasonable doubt as to the enablement of the entire scope of the claim, the specification itself may contain sufficient support for the full scope even in an unpredictable art. *Ex parte Singh* , 17 USPQ2d 1714 (BPAI 1991), *In re Goodman* 29 USPQ2d 2010 (CA FC 1993) and *In re Vaeck*, 20 USPQ2d 1438 (CA FC). "We do not imply that patent applicants in art areas currently denominated as 'unpredictable' must never be allowed generic claims encompassing more than the particular species disclosed in their specification. It is well settled that patent applicants are not required to disclose every species encompassed by their claims, even in an unpredictable art." *In re Vaeck, at 1445, citing In re Angstadt, 190 USPQ 214, 218 (CCPA 1976).* Thus, scope of enablement can be overcome with the proper disclosure even if the art is considered unpredictable.

The Washington Post article describes a woman who died and "[h]er decline had begun the day after her right knee was injected with an experimental drug made of genetically engineered viruses." However, the article noted, "[n]o one knows yet whether the treatment was to blame. Of the dozens of other volunteers who got the injections, only Mohr suffered anything more than short-lived side effects[.]" The article primarily focuses on possible "[b]reaches of clinical research standards and a federal oversight system[.]" There is little to no technical information provided in the article other than that "an experimental drug made of genetically

engineered viruses" was used. Further, it has since been reported that the woman's death was *not* caused by this experimental drug. "The U.S. Food and Drug Administration has allowed Targeted Genetics to restart its gene-therapy trial for rheumatoid arthritis after an investigation indicated the treatment did not contribute to the death of an Illinois woman in July." *See* http://seattletimes.nwsource.com/cgi-bin/PrintStory.pl?document_id=2004035019&zsection_id=2003907475&slug=targetedgenetics26&date=20071126 (last visited February 2, 2008) for a recent report on this issue, copy provided *herewith* as Exhibit 1.

Furthermore, whether or not the Examiner has successfully demonstrated "unpredictability" based on this reference is not controlling, because the present specification has enabled the claimed invention whether or not the art is "unpredictable." The present application has provided working examples in which Her-2/neu DNA vaccines were effective at providing antitumor immunity against human Her-2/neu expressing murine tumor cells (Her-2-CT26) in mice. Thus, none of the evidence or arguments set forth by the Examiner established that immune correlates of the present invention are "unbelievable" or "unpredictable."

Patent law only requires a showing that "reasonable correlation" exists between the scope of the claims and the scope of enablement. As stated in the M.P.E.P. § 2164.02, "'correlation' as used herein refers to the relationship between *in vitro* or *in vivo* animal model assays and a disclosed or a claimed method of use." If a particular model is recognized as correlating to a specific condition, then it should be accepted as such unless the Examiner has evidence that the model does not correlate. *In re Brana*, 51 F.3d 1560, (Fed. Cir. 1995), at 1566. Here the specification has provided working examples in an established animal model. Further, "DNA vaccination has proven to be a generally applicable approach to various pre-clinical animal

models of infectious and non-infectious diseases, and several DNA vaccines have now entered phase I/II human clinical trials." *See* Bocchia *et al*, Haematologica 85: 1172-1206, 1185, 2000.

According to the Examiner's apparent view of the scope of enablement requirement, an Applicant would have to submit conclusive data from human clinical trials in order to adequately enable a method of treatment applicable to humans. This is clearly in conflict with the statute, the rules and the guidelines of the M.P.E.P. Specifically, under the current case law, clinical efficacy is not required to show that a therapeutic process is operable. As stated in M.P.E.P. § 2107.01, the "courts have found utility for therapeutic inventions, despite the fact that an applicant is at a very early stage in the development of a therapeutic regimen" or that a therapeutic treatment regimen is not at a stage where it is ready to be practiced on humans. *Cross v. Iizuka*, 753 F.2d 1040, 224 U.S.P.Q. 739 (Fed. Cir. 1985); *In re Brana*, 51 F.3d 1560, 34 U.S.P.Q.2d 1436 (Fed. Cir. 1995).

It is not within the province of the USPTO to require proof of efficacy in animals, let alone humans, to grant a patent including claims to therapeutic methods. The FDA will accept evidence from animal studies to provide substantial evidence of the effectiveness of products, when human efficacy studies are not feasible. For the FDA approval purposes it is sufficient to establish the effect in either more than one animal, or in a single well-characterized animal model that has been shown to predict the human response. *See* 67 Fed. Reg. 37989, May 31, 2002 (attached *herewith* as Exhibit 2).

The PTO guidelines do not require proof of efficacy, and are, in fact, explicit on this point: "Office personnel should not impose on applicants the unnecessary burden of providing evidence from human clinical trials. There is no decisional law that requires an applicant to provide data from human clinical trials to establish utility for an invention related to treatment of

human disorders." M.P.E.P. § 2107.03. The guidelines further state that "[t]he Office must confine its review of patent applications to the statutory requirements of the patent law, and in quoting *In re Brana, supra*, that "FDA approval, however, is not a prerequisite for finding a compound useful within the meaning of the patent laws". *Id.* In fact, all that is required by the patent laws is that a "reasonable correlation" exists between the scope of the claims and the scope of enablement. Citing to M.P.E.P. § 2164.02. As stated in *Cross v. Iizuka, supra*, at 1050, a rigorous or an invariable exact correlation is not required. Applicant asserts that a reasonable correlation thus exists between the data provided in the captioned application and the claimed methods.

The Examiner has not provided any reason to doubt the truth of the statements concerning the prophylactic effect using the claimed methods and composition as disclosed in the specification or the acceptance of the mouse model as correlating to protective immunity in humans. Thus, for the reasons given above, Applicant submits that the scope of the present claims is commensurate in scope with the enablement provided in the present specification. The considerations raised by the Examiner either are resolved by the teachings in the specification or would have required only routine experimentation by one of skill in the art to practice the claimed invention.

Further, a post-filing manuscript (attached *herewith* as Exhibit 3), co-authored by an inventor of the captioned application, reports that primate studies showed that "genetic vaccines efficiently elicited Her-2/neu-specific humoral and cellular immune responses without causing severe adverse effects in non-human primates[.]" See Exhibit 3, page 2. A pCK bicistronic truncated Her-2/neu and GM-CSF construct of the current invention was tested in primates. *See* Specification at [0064] - [0068]; [0121] - [0124]. To assess the efficacy and the safety of

truncated anti-Her-2/neu genetic vaccines in large animals, rhesus monkeys (*Macaca mulatta*) were immunized with DNA vaccine and/or adenovirus vaccine. The results support that Her-2/neu genetic vaccines are immunogenic and safe in primates. Applicants assert that this post-filing data further supports the correlation that exists between the data provided in the captioned application and the claimed methods.

Thus, given the explicit disclosure of specific *in vivo* working examples in the specification using a model that reasonably correlate to mammals, including humans, and post-filing art showing the efficacy of truncated Her-2/neu DNA vaccines in primates, Applicants respectfully submit that one skilled in the art would be able to make and use the claimed invention without undue experimentation. Accordingly, Applicants request reconsideration and withdrawal of the enablement rejection in view of the amendment to the claims and the remarks herein.

35 U.S.C. § 112, second paragraph

The Examiner has rejected claim 25 under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. OA at page 9. Specifically, the Examiner asserts that claim 25 does not recite the method step that is to be applied and that "the meaning of "after tumor surgery" is unclear." OA at pages 9. Claim 25 has been canceled. Insofar that the rejection applies to the currently pending claim 56, Applicants have reworded the claim to recite the method step and clarified the phrase "after tumor surgery." Thus, Applicants respectfully request that this rejection be reconsidered and withdrawn.

Rejections under 35 U.S.C. § 103(a)

Piechoki and Lee, as evidenced by Bocchia

The Examiner has rejected claims 1-3, 13, 16, and 19-28 under 35 U.S.C. § 103(a) as being obvious over Piechocki *et al.* (J. Immunol. 167: 3367-3374, 2001, hereinafter "Piechocki") and Lee *et al.* (Biochem. Biophys. Res. Comm. 272(1): 230-235, 2000, hereinafter "Lee"), as evidenced by Bocchia *et al.* (Haematologica 85: 1172-1206, 2000, hereinafter "Bocchia"). OA at pages 9-13. More specifically, the Examiner asserts that Piechocki teaches use of a plasmid DNA vaccine comprising a nucleotide sequence encoding a truncated human Her-2/neu polypeptide, specifically the terminal amino acids 1-505 of the mature human Her-2/neu extracellular domain. OA at page 10. Further, Examiner asserts that Piechocki teaches a method of preventing or treating cancer. OA at pages 10-12. Examiner asserts that Lee taught the construction of a pCK expression plasmid that is able to drive high levels of expression *in vivo* for therapeutic use. OA at page 12. The Examiner asserts that Bocchia teaches that in the real world clinical setting, human patients will present with tumor disease before being therapeutically treated with a tumor vaccine. OA at page 11. Claims 1-3, 13, 16, and 19-28 have been cancelled. Insofar that the rejection applies to the currently pending claims 29-60, Applicants respectfully traverse this rejection.

The legal standard

The factors to be considered under 35 U.S.C. § 103(a) are the scope and content of the prior art; the differences between the prior art and the claims at issue; and the level of ordinary skill in the pertinent art. *See Graham v. John Deere*, 86 S.Ct. 684 (1966) and MPEP §2141. This analysis has been the standard for 40 years, and remains the law today. *See KSR*

International Co v. Teleflex Inc., 127 S.Ct. 1727 (2007). The Office has recently published Examination Guidelines to aid Examiners in formulating obviousness rejections. *See Examination Guidelines for Determining Obviousness under 35 U.S.C. 103 in view of the Supreme Court decision in KSR International v. Teleflex Inc. Fed.* Reg. Vol. 72, pp. 57526 to 57535 (October 10, 2007), hereinafter "the Examination Guidelines." Seven rationales are suggested by which obviousness may be found, *e.g.*, by combining elements in the art or substituting one known element for another. As a common thread through all the rationales, the Examiner must establish on the record that a person of ordinary skill in the art would have recognized that the results of the combination or substitution were *predictable*. *Id.*, e.g., at 57529.

The Applicants' arguments

The Examiner impermissibly rejects the claims as being obvious over the cited art while at the same time asserting that the art in the area of DNA vaccines in general was unpredictable, and was especially unpredictable in reference to DNA vaccines in humans. OA at pages 4-9. Art that is deemed unpredictable cannot be properly combined to reject a claim under 35 U.S.C. § 103(a) as being obvious, because establishment of a *prima facie* case of obviousness requires a factual showing that the combination is *predictable.*

Initially, in order to support a *prima facie* case of obviousness, the prior art must suggest making the specific molecular modifications necessary to achieve the claimed invention. *See In re Deuel*, 51 F.3d 1552, 1558 (Fed. Cir. 1995); *In re Lalu*, 747 F.2d 703, 705 (Fed. Cir. 1984) ("[t]he prior art must provide one of ordinary skill in the art the motivation to make the proposed molecular modifications needed to arrive at the claimed compound."). That is, simply because "one can conceive a general process in advance for preparing an *undefined* compound does not

mean that a claimed *specific* compound was precisely envisioned and therefore obvious." *See In re Deuel* at 1559. In order for the cited reference to be suitable as a primary reference upon which to base a *prima facie* case of obviousness, it must be *predictable* that the artisan would arrive at the specifically claimed plasmid construct. Here, the claimed truncated Her-2/neu plasmid construct and methods for preventing or treating Her-2/neu-overexpressing cancers with said plasmid construct were not precisely envisioned in the prior art, especially in view of the numerous potential Her-2/neu plasmid constructs that could be envisioned, and may or may not be effective. Therefore, the cited references taken together are seriously deficient (particularly in view of the holding in *Deuel*), and cannot support a *prima facie* case of obviousness.

Claims 29-60 are directed to compositions and methods related to a pTV2 or pCK plasmid construct comprising a nucleotide sequence encoding a C-terminally truncated human Her-2/neu protein consisting essentially of the *entire* extracellular domain and transmembrane domain of Her-2/neu, or the *entire* extracellular domain of Her-2/neu. Applicants assert that Piechocki and Lee do not teach or suggest the compositions or methods recited in the claims.

Piechocki describes a pCMV plasmid with a nucleotide sequence encoding a secreted Her-2/neu (secE2) that includes the ER signal peptide (aa 1-21) and N-terminal amino acids 1-505 (encoding most of the ECD) of the mature protein for use as a DNA vaccine. Piechocki at page 3368. Unlike the current invention, the Piechocki construct does not contain the *entire* extracellular domain and does not include the transmembrane domain. At the time of Applicants' invention, it was known that oncogenic activity prohibited use of full-length Her-2/neu as a DNA vaccine. *See* Piechocki at 3367. However, it was *unknown* which Her-2/neu epitopes were likely to provide the best tumor rejection in murine and human models. As stated in Piechocki, "there is still some debate as to which epitopes are most important for tumor

rejection in murine and human models and the way in which to best present these Ags to the immune system for effective priming." *See* Piechocki at 3369.

Piechocki disclosed that even a single residue substitution in full-length Her-2/neu (E2A) greatly reduced efficacy. *See* Piechocki at 3367. Therefore, a person of ordinary skill in the art after reading Piechocki would have had no guidance regarding whether a pTV2 or pCK plasmid construct comprising a nucleotide sequence encoding C-terminally truncated Her-2/neu that includes the *entire* Her-2/neu extracellular domain or the *entire* extracellular domain and the transmembrane domain would provide sufficient anti-tumor activity to support use as an effective preventative or therapeutic DNA vaccine.

Further, Piechocki also tested a pCMV Her-2/neu construct that included a truncated endoplasmic reticulum (ER) domain, but intact intracellular domain, extracellular domain and transmembrane domain (cytE2), and an lysine to alanine mutation of this construct (cytE2A). These are the exact same constructs described by Pilon and are discussed below.

All of the Her-2/neu plasmids described in Piechocki were constructed using a pCMV vector. Piechocki provides no disclosure as to the effect alternative plasmid vectors would have on the ability of a nucleotide sequence encoding a truncated human Her-2/neu protein to provide sufficient tumor immunity to support use as an effective DNA vaccine. A person of ordinary skill would find no guidance in Piechocki regarding pCK or pTV2 vectors for expressing a nucleotide sequence encoding truncated Her-2/neu for use as a DNA vaccine.

Lee discloses a pCK expression vector that is able to drive high levels of $VEGF_{165}$ gene expression in the skeletal muscles of mice. Lee does not describe a pTV2 or pCK expression vector that includes Her-2/neu DNA. The Examiner is reminded that in order for the cited reference to be suitable as a primary reference upon which to base a *prima facie* case of

obviousness, it must be *predictable* that the artisan would arrive at the specifically claimed pTV2 or pCK plasmid construct comprising a nucleotide sequence encoding a C-terminally truncated human Her-2/neu protein consisting essentially of the entire extracellular domain and transmembrane domain of Her-2/neu, or the entire extracellular domain of Her-2/neu.

Applicants assert that the plasmid construct of the invention was not predictable in view of the cited references, especially in view of the numerous potential vectors that could be envisioned. As Examiner points out, "results of tumor antigen DNA vaccine approaches might be improved by optimization of key variables such as dosage, route, vector design, and boosting strategies." OA at pages 4-5. Lee provides no guidance for use of the pCK vector with nucleotide sequences other than $VEGF_{165}$. $VEGF_{165}$ and Her-2/neu are not structurally or functionally similar. $VEGF_{165}$ and Her-2/neu do not share similar nucleotide sequences and the proteins encoded by these nucleotide sequences are not described as sharing similar functions. $VEGF_{165}$ gene has been shown to be effective in ischemic diseases including coronary artery disease and peripheral arterial occlusive disease. *See* Lee at 230. Her-2/neu gene is known to be amplified and overexpressed in several types of human adenocarcinomas. *See* Specification at page 1, lines 15-28. Therefore, the cited references taken together are seriously deficient (particularly in view of the holding in *Deuel*), and cannot support a *prima facie* case of obviousness.

The Examiner alleges that although "Piechocki et al do not teach the DNA vaccination method to be performed after a method step of tumor surgery," Bocchia "recognized that in the real world clinical setting, human patients will present with tumor diseases before being therapeutically treated with a tumor vaccine." OA at page 11. Bocchia does not teach or suggest a composition, specifically a pTV2 or pCK plasmid construct comprising a nucleotide

sequence encoding a C-terminally truncated human Her-2/neu protein consisting essentially of the entire extracellular domain and transmembrane domain of Her-2/neu, or the entire extracellular domain of Her-2/neu as required by the present claims. Thus, the references do not suggest a composition, let alone the methods of the claimed invention.

The deficiencies of Piechocki and Lee, as evidenced by Bocchia have been discussed above. The combined references cited by the Examiner do not suggest the specifically claimed C-terminally truncated Her-2/neu plasmid construct or methods of the claimed invention. Finally, a person of ordinary skill in the art would not predictably arrive at the claimed invention upon combination of the references, given the unpredictability of the art as described above. Therefore, Applicants respectfully request withdrawal of the rejection as it relates to the currently pending claims.

Piechoki and Lee, in further view of Steinaa and Pilon

The Examiner has rejected claims 1, 11-15 and 17-18 under 35 U.S.C. § 103(a) as being obvious over Piechocki and Lee, in further view of Steinaa *et al.* (U.S. Patent No. 7,005,498 B1, hereinafter "Steinaa") and Pilon et al. (J. Immunol. 167: 3201-3206, 2001, hereinafter "Pilon"). OA at pages 13-15. More specifically, the Examiner asserts that "[Steinaa] et al contemplated a DNA vaccine composition comprising a nucleic acid vector encoding a human Her-2/neu polypeptide, wherein the Her-2/neu polypeptide may lack the intracellular domain." OA at page 14. Examiner also alleges that "Stienna et al also contemplated the DNA vaccine composition to comprise the cytokine GM-CSF." OA at page 14. Similarly, Examiner alleges "Pilon et al taught a DNA vaccine composition comprising a nucleic acid encoding a human Her-2/neu polypeptide, wherein the composition further comprised a plasmid expressing the GM-CSF cytokine." OA at page 14. Claims 1, 11-15 and 17-18 have been canceled. Insofar that the

rejection applies to the currently pending claims 29-60, Applicants respectfully traverse this rejection.

The deficiencies of Piechocki and Lee have been discussed above. Further, Steinaa and Pilon do not cure the deficiencies of Piechocki and Lee. Specifically, Steinaa describes DNA vaccines generally. Steinaa at col. 24, lines 45-50. Further, Steinaa references Her2/neu as an example of an EGFr family member where overexpression has been closely linked with poor prognosis in cancers. Steinaa at col. 31, lines 30-33; cols. 39-40. Finally, Steinaa discloses a prophetic example where "human autovaccine against Her-2 can be developed through modification of the molecule by insertion of one or more promiscuous foreign T cell epitopes to reveal a panel of immunogenised Her-2 molecules." Steinaa at cols. 65-67. The disclosure in Steinaa does not provide any guidance for a person of ordinary skill in the art to make or use a pTV2 or pCK plasmid construct comprising a nucleotide sequence encoding a C-terminally truncated human Her-2/neu protein consisting essentially of the entire extracellular domain and transmembrane domain of Her-2/neu, or the entire extracellular domain of Her-2/neu. As discussed above, given the unpredictability of the art, it would *not* have been obvious, given the numerous potential Her-2/neu plasmid constructs that could be envisioned, which Her-2/neu plasmid constructs would provide preventative or therapeutic anti-tumor immunity in a mammal. Although Steinaa generally mentions cytokines, including GM-CSF, as adjuvants, the compositions and methods of the present invention are not described or taught by Steinaa alone or in combination with the other references cited by the examiner. Steinaa at col. 17, lines 24-33; col. 25, lines 52-56. The Examiner is reminded that simply because "one can conceive a general process in advance for preparing an *undefined* compound does not mean that a claimed *specific* compound was precisely envisioned and therefore obvious." *See In re Deuel* at 1559.

Pilon discloses pCMV Her-2/neu constructs, cytE2 and cytE2A, which encode "full-length protein that is targeted to and rapidly degraded in the cytosol by the proteoasomes." *See* Pilon at 3201. Unlike the current invention, the Pilon constructs have a truncated endoplasmic reticulum (ER) domain, but intact intracellular domain, extracellular domain and transmembrane domain (cytE2). Therefore, these constructs are not C-terminally truncated and lack a signal peptide. The Pilon constructs were also described in Piechocki. As discussed in Pilon and Piechocki, these constructs showed reduced preventative efficacy against D2F2/E2 tumors in mice as compared to full-length Her-2/neu (E2). *See* Pilon at page 3205. Vaccination with E2, E2A, cytE2, or cytE2A resulted in ~90, 60, 30, and 10% protection against D2F2/E2 tumor, respectively[.]" *Id.* The recombinant proteins all contained the same, complete structural sequence of E2, but their immunogenicity was affected by the subcellular localization, membrane stability, and signaling activity of the recombinant protein." *See* Piechocki at 3367.

Further, no therapeutic efficacy was demonstrated in either Piechocki or Pilon. In contrast, the above captioned application discloses a C-terminally truncated Her-2/neu that includes the *entire* Her-2/neu extracellular domain or the *entire* extracellular domain and the transmembrane domain which has both high preventative and therapeutic anti-tumor activity. *See* Specification at [0031] - [0039], Figures 10-16, and [0060]. A person of ordinary skill in the art after reading the Pilon (or Piechocki) disclosure would not have the guidance needed to make the specific molecular modifications necessary to achieve the claimed invention. *See In re Deuel*, 51 F.3d 1552, 1558 (Fed. Cir. 1995) Thus, the Examiner has failed to present a *prima facie* case of obviousness, the prior art does not suggest a composition or methods related to a pTV2 or pCK plasmid construct comprising a nucleotide sequence encoding a C-terminally

truncated human Her-2/neu protein consisting essentially of the entire extracellular domain and transmembrane domain of Her-2/neu, or the entire extracellular domain of Her-2/neu.

As such, the combined references cited by the Examiner do not suggest the claimed methods, and specifically claimed compositions. Finally, a person of ordinary skill in the art would not predictably arrive at the claimed invention upon combination of the references, given the unpredictability of the art as described above. Therefore, Applicants respectfully request withdrawal of the rejection as it relates to the currently pending claims.

Conclusion

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Applicants therefore respectfully request that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. Applicants believe that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,

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EXHIBIT 1

The Seattle Times
seattletimes.com

Monday, November 26, 2007 - Page updated at 12:00 AM

Targeted Genetics restarts trial after woman's death

Targeted Genetics CEO H. Stewart Parker

By Ángel González
Seattle Times business reporter

The U.S. Food and Drug Administration has allowed Targeted Genetics to restart its gene-therapy trial for rheumatoid arthritis after an investigation indicated the treatment did not contribute to the death of an Illinois woman in July.

The trial was put on hold when 36-year old Jolee Mohr died of a massive fungal infection weeks after being injected with the second dose of a treatment designed to fight arthritis by weakening the immune system in targeted areas.

A post-mortem analysis by University of Chicago researchers concluded that the Seattle-based company's drug didn't wildly spread through the patient's body, nor did it significantly increase the immune suppression produced by another anti-arthritis drug she was taking.

The FDA's decision clears some concern about the future of gene therapy, a field considered promising but risky. It also helps restore confidence in Target Genetics' clinical pipeline, which relies on gene-therapy products.

"This is very good for the company, for the product and for gene therapy," said Chief Executive H. Stewart Parker.

But Mohr's death also raised issues about whether patients with nonlethal diseases were being properly informed of the risks of medical experimentation before consenting to participate in clinical trials.

Targeted Genetics said that it was revising the "informed consent" document patients review before enrolling to include information about Mohr's death. The company is also amending the study's procedure to keep doctors from administering the drug to patients suffering from fever at the time scheduled for the injection. Mohr had a mild fever when she was injected with the treatment.

The study involves 127 patients, some 35 of whom are yet to receive a second dose of the treatment.

Over the next few weeks, Targeted Genetics will again seek consent from those patients and the approval of an independent ethics review board for the amended protocol.

"We'll certainly be up in the first quarter of the year," Parker said.

The company plans to start a follow-up, midstage clinical trial by mid- to late 2008.

Targeted Genetics' product relies on a tame virus — "adeno-associated virus," or AAV — to carry an immunosuppressant gene to selected areas of the body, such as the joints.

The gene helps block tumor necrosis factor-a, or TNFa, a protein that in excess amounts causes severe swelling and pain.

Targeted Genetics' therapy was being tested as a complement to other anti-TNFa drugs that didn't prove effective enough at reducing the symptoms of the disease in some patients.

It was feared that the injection of Targeted Genetics' product, in combination with the therapy Mohr was already taking, might have helped overwhelm the patient's immune system.

But molecular analysis of the patient's tissue found only traces of the AAV outside her joints, and the level of anti-TNFa blockage was typical of a patient taking general rheumatoid-arthritis treatments.

Histoplasmosis — the infection that killed Mohr — is a known side effect of some of these therapies, although most cases are mild.

A National Institutes of Health panel is scheduled to meet next month to discuss results of the investigation of Mohr's death.

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EXHIBIT 2

d.6. Silicon carbide;
d.7. Tantalum or tantalum alloys;
d.8. Titanium or titanium alloys;
d.9. Titanium carbide; *or*
d.10. Zirconium or zirconium alloys.
e. Distillation or absorption columns of internal diameter greater than 0.1 m, and liquid distributors, vapor distributors or liquid collectors designed for such distillation or absorption columns, where all surfaces that come in direct contact with the chemical(s) being processed are made from any of the following materials:
e.1. Alloys with more than 25% nickel and 20% chromium by weight;
e.2. Fluoropolymers;
e.3. Glass (including vitrified or enamelled coatings or glass lining);
e.4. Graphite or carbon-graphite;
e.5. Nickel or alloys with more than 40% nickel by weight;
e.6. Tantalum or tantalum alloys;
e.7. Titanium or titanium alloys; *or*
e.8. Zirconium or zirconium alloys.
f. Remotely operated filling equipment in which all surfaces that come in direct contact with the chemical(s) being processed are made from any of the following materials:
f.1. Alloys with more than 25% nickel and 20% chromium by weight; *or*
f.2. Nickel or alloys with more than 40% nickel by weight.
g. Valves with nominal sizes greater than 1.0 cm (3/8 in.), in which all surfaces that come in direct contact with the chemical(s) being processed or contained are made from any of the following materials:
g.1. Nickel or alloys with more than 40% nickel by weight;
g.2. Alloys with more than 25% nickel and 20% chromium by weight;
g.3. Fluoropolymers;
g.4. Glass or glass lined (including vitrified or enameled coatings);
g.5. Tantalum or tantalum alloys;
g.6. Titanium or titanium alloys; *or*
g.7. Zirconium or zirconium alloys.
h. Multi-walled piping incorporating a leak detection port, in which all surfaces that come in direct contact with the chemical(s) being processed or contained are made from any of the following materials:
h.1. Alloys with more than 25% nickel and 20% chromium by weight;
h.2. Fluoropolymers;
h.3. Glass (including vitrified or enamelled coatings or glass lining);
h.4. Graphite or carbon-graphite;
h.5. Nickel or alloys with more than 40% nickel by weight;
h.6. Tantalum or tantalum alloys;
h.7. Titanium or titanium alloys; *or*
h.8. Zirconium or zirconium alloys.
i. Multiple-seal, canned drive, magnetic drive, bellows or diaphragm pumps, with manufacturer's specified maximum flow-rate greater than 0.6 m^3/hour, or vacuum pumps with manufacturer's specified maximum flow-rate greater than 5 m^3/hour (under standard temperature (273 K (0° C)) and pressure (101.3 kPa) conditions), and casing (pump bodies), preformed casing liners, impellers, rotors or jet pump nozzles designed for such pumps, in which all surfaces that come into direct contact with the chemical(s) being processed are made from any of the of the following materials:
i.1. Alloys with more than 25% nickel and 20% chromium by weight;
i.2. Ceramics;
i.3. Ferrosilicon;
i.4. Fluoropolymers;
i.5. Glass (including vitrified or enamelled coatings or glass lining);
i.6. Graphite or carbon-graphite;
i.7. Nickel or alloys with more than 40% nickel by weight;
i.8. Tantalum or tantalum alloys;
i.9. Titanium or titanium alloys, *or*
i.10. Zirconium or zirconium alloys.
j. Incinerators designed to destroy chemical warfare agents, chemical weapons precursors controlled by 1C350, or chemical munitions having specially designed waste supply systems, special handling facilities and an average combustion chamber temperature greater than 1000°C in which all surfaces in the waste supply system that come into direct contact with the waste products are made from or lined with any of the following materials:
j.1. Alloys with more than 25% nickel and 20% chromium by weight;
j.2. Ceramics; *or*
j.3. Nickel or alloys with more than 40% nickel by weight.

Technical Note: Carbon-graphite is a composition consisting primarily of graphite and amorphous carbon, in which the graphite is 8 percent or more by weight of the composition.

19. In Supplement No. 1 to Part 774 (the Commerce Control List), Category 2—Materials Processing, is amended by revising the List of Items Controlled section in ECCN 2B352 to read as follows:

2B352 Equipment capable of use in handling biological materials, as follows (see List of Items Controlled).

* * * * *

List of Items Controlled

Unit: Equipment in number.
Related Controls: N/A.
Related Definitions: For purposes of this entry, isolators include flexible isolators, dry boxes, anaerobic chambers and glove boxes.
Items:
a. Complete containment facilities at P3 or P4 containment level.

Technical Note: P3 or P4 (BL3, BL4, L3, L4) containment levels are as specified in the WHO Laboratory Biosafety Manual (Geneva, 1983).

b. Fermenters capable of cultivation of pathogenic microorganisms, viruses, or for toxin production, without the propagation of aerosols, having a capacity equal to or greater than 100 liters.

Technical Note: Fermenters include bioreactors, chemostats, and continuous-flow systems.

c. Centrifugal separators capable of the continuous separation of pathogenic microorganisms, without the propagation of aerosols, and having all of the following characteristics:
c.1. One or more sealing joints within the steam containment area;
c.2. A flow rate greater than 100 liters per hour;
c.3. Components of polished stainless steel or titanium; *and*
c.4. Capable of *in situ* steam sterilization in a closed state.

Technical Note: Centrifugal separators include decanters.

d. Cross (tangential) flow filtration equipment capable of continuous separation of pathogenic microorganisms, viruses, toxins, and cell cultures without the propagation of aerosols, having all of the following characteristics:
d.1. Equal to or greater than 5 square meters;
d.2. Capable of *in situ* sterilization.
e. Steam sterilizable freeze-drying equipment with a condenser capacity of 10 kgs of ice or greater in 24 hours, but less than 1,000 kgs of ice in 24 hours.
f. Protective and containment equipment, as follows:
f.1. Protective full or half suits, or hoods dependant upon a tethered external air supply and operating under positive pressure;

Technical Note: This entry does not control suits designed to be worn with self-contained breathing apparatus.

f.2. Class III biological safety cabinets or isolators with similar performance standards, *e.g.*, flexible isolators, dry boxes, anaerobic chambers, glove boxes or laminar flow hoods (closed with vertical flow).
g. Chambers designed for aerosol challenge testing with microorganisms, viruses, or toxins and having a capacity of 1 m^3 or greater.

Dated: May 23, 2002.

James J. Jochum,

Assistant Secretary for Export Administration.

[FR Doc. 02–13581 Filed 5–30–02; 8:45 am]
BILLING CODE 3510–33–P

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration

21 CFR Parts 314 and 601

[Docket No. 98N–0237]

RIN 0910–AC05

New Drug and Biological Drug Products; Evidence Needed to Demonstrate Effectiveness of New Drugs When Human Efficacy Studies Are Not Ethical or Feasible

AGENCY: Food and Drug Administration, HHS.

ACTION: Final rule.

SUMMARY: The Food and Drug Administration (FDA) is amending its new drug and biological product regulations to allow appropriate studies in animals in certain cases to provide

substantial evidence of the effectiveness of new drug and biological products used to reduce or prevent the toxicity of chemical, biological, radiological, or nuclear substances. This rule will apply when adequate and well-controlled clinical studies in humans cannot be ethically conducted and field efficacy studies are not feasible. In these situations, certain new drug and biological products that are intended to reduce or prevent serious or life-threatening conditions may be approved for marketing based on evidence of effectiveness derived from appropriate studies in animals and any additional supporting data.

DATES: This rule is effective July 1, 2002.

FOR FURTHER INFORMATION CONTACT:
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SUPPLEMENTARY INFORMATION:

I. Introduction

In the **Federal Register** of October 5, 1999 (64 FR 53960), we (FDA) proposed to amend our new drug and biological product regulations to identify the information needed to provide substantial evidence of the effectiveness of certain new drug and biological products used to reduce or prevent the toxicity of chemical, biological, radiological, or nuclear substances. We are finalizing that proposed rule by adding subpart I to part 314 (21 CFR part 314) and subpart H to part 601 (21 CFR part 601).

This final rule provides for approval of certain new drug and biological products based on animal data when adequate and well-controlled efficacy studies in humans cannot be ethically conducted because the studies would involve administering a potentially lethal or permanently disabling toxic substance or organism to healthy human volunteers and field trials are not feasible prior to approval. Under this rule, in these situations, certain new drug and biological products that are intended to reduce or prevent serious or life-threatening conditions can be approved for marketing based on evidence of effectiveness derived from appropriate studies in animals, without adequate and well-controlled efficacy studies in humans (§ 314.126). In assessing the sufficiency of animal data, the agency may take into account other data, including human data, available to the agency. Under this rule, FDA can rely on the evidence from animal studies to provide substantial evidence of the effectiveness of these products when:

1. There is a reasonably well-understood pathophysiological mechanism for the toxicity of the chemical, biological, radiological, or nuclear substance and its amelioration or prevention by the product;

2. The effect is demonstrated in more than one animal species expected to react with a response predictive for humans, unless the effect is demonstrated in a single animal species that represents a sufficiently well-characterized animal model (meaning the model has been adequately evaluated for its responsiveness) for predicting the response in humans;

3. The animal study endpoint is clearly related to the desired benefit in humans, which is generally the enhancement of survival or prevention of major morbidity; and

4. The data or information on the pharmacokinetics and pharmacodynamics of the product or other relevant data or information in animals and humans is sufficiently well understood to allow selection of an effective dose in humans, and it is therefore reasonable to expect the effectiveness of the product in animals to be a reliable indicator of its effectiveness in humans.

All studies subject to this rule must be conducted in accordance with preexisting requirements under the good laboratory practices (21 CFR part 58) regulations and the Animal Welfare Act (7 U.S.C. 2131 *et. seq.*).

Safety evaluation of products is not addressed in this rule. Products evaluated for effectiveness under subpart I of part 314 and subpart H of part 601 will be evaluated for safety under preexisting requirements for establishing the safety of new drug and biological products. The agency believes that the safety of most of these products can be studied in human volunteers similar to the people who would be exposed to the product. FDA recognizes that some safety data, such as data on possible adverse interactions between the toxic substance itself and the new product, may not be available. This is not expected to keep the agency from making an adequate safety evaluation. FDA's procedures and standards for evaluating the safety of new drug and biological products are sufficiently flexible to provide for the safety evaluation of products evaluated for efficacy under subpart I of part 314 and subpart H of part 601.

This rule will not apply if product approval can be based on standards described elsewhere in our regulations (for example, accelerated approval based on human surrogate markers or clinical endpoints other than survival or irreversible morbidity).[1]

II. Comments on the Proposed Rule and Our Response

We received comments on the proposed rule from two pharmaceutical companies and one physician affiliated with a university. We also received comments from the National Institutes of Health (NIH). The NIH comments were based on a prepublication draft of the proposed rule, but the comments were received too late to be addressed in the proposed rule. The NIH comments have been placed in the docket for this rule and are addressed in this document.

In addition to the changes we have made in response to comments, we have changed the titles of subpart I of part 314 and subpart H (formerly subpart G) of part 601 to better describe the scope of the subparts. Subpart I of part 314 is now entitled "Approval of New Drugs When Human Efficacy Studies Are Not Ethical or Feasible" and subpart H of part 601 is now entitled "Approval of Biological Products When Human Efficacy Studies Are Not Ethical or Feasible." Proposed subpart G has been redesignated as subpart H in the final rule because subpart G has since been designated for regulations on postmarketing studies. Proposed §§ 601.60 through 601.65 have been renumbered §§ 601.90 through 601.95 in subpart H.

We have also changed, on our own initiative, the requirements proposed in §§ 314.610(c) and 601.61(c) (§§ 314.610(b)(3) and 601.91(b)(3) in this final rule). We have deleted the requirement that self-administered drug products approved under this rule be in unit-of-use packaging with attached patient labeling. In addition, we have eliminated the distinction between self-

[1] An example of a drug approval based on human surrogate markers is our August 30, 2000, approval of an efficacy supplement for ciprofloxacin. Ciprofloxacin HCl was approved for postexposure management of inhalational anthrax. The approval was based, in part, on human studies demonstrating that ciprofloxacin achieved serum concentrations reaching or exceeding levels associated with improved survival of animals exposed to aerosolized *Bacillus anthracis* spores. The results from these studies were combined with the knowledge of effectiveness in humans of ciprofloxacin for other bacterial infections, including pneumonia. The validity of the human surrogate marker was supported by animal studies.

administered products and products administered by health professionals.

Whether a product is self-administered or administered by a health professional, it is important to inform patient recipients that a product approved under this rule has not been studied for efficacy in humans because of ethical or feasibility reasons.[2] It is also important that patient recipients receive information about indications, dosage and administration, contraindications, reasonably foreseeable risks, adverse reactions, anticipated benefits, and drug interactions. This rule requires that all of this information be provided to patient recipients of products approved under subpart I of part 314 and subpart H of part 601.

We believe, however, that the proposed unit-of-use packaging and attached patient-labeling requirement could have had the unintended effect of hampering the distribution and dispensing of these products in the event of an emergency. The added bulk of unit-of-use packaging could have made stockpiling and transporting more difficult in many cases. The proposed requirement might also have hampered the speedy distribution of products for additional indications previously approved outside of this rule.

Applicants may meet the requirements of new §§ 314.610(b)(3) and 601.91(b)(3) in a variety of ways, as long as sponsors make provisions to get the information to patients. For example, the sponsor could provide reproducible master copies of labeling information or presentations for patient recipients that would be appropriate in the event of an emergency.

We have also changed proposed §§ 314.610(c) and 601.61(c) (§§ 314.610(b) and 601.91(b) in this final rule) to require that the patient labeling explain that, for ethical or feasibility reasons, the product's approval was based on efficacy studies conducted only in animals. This explanation will better inform patient recipients about the nature and ethical basis of the product approval under this rule and how that approval differs from approval of products based on standard human efficacy studies.

Finally, we have added to §§ 314.610(b)(1) and 601.91(b)(1) (proposed §§ 314.610(a) and 601.61(a)) a requirement that applicants include a plan or approach to fulfilling postmarketing study commitments as part of their application. We recognize that such studies normally will not be conducted unless an emergency arises that requires the product's use. Furthermore, when the product is used in an emergency, it may not be feasible for sponsors to conduct postmarketing studies in a timely manner, nor is it our intention to require sponsors to send investigators into areas of exposure. We do, however, believe that applicants can plan a postmarketing study approach, in consultation with the agency, as part of an overall response to an event.

The requirement to submit a plan for postmarketing studies is consistent with the requirements for sponsors under the accelerated approval process provided for in subpart H of part 314.

The procedures in subpart H and in this rule are similar because, to assess efficacy, both allow use of an endpoint that is not a clinical endpoint showing a benefit. Instead the rules under subpart H allow for reliance on a clinical surrogate endpoint and this rule allows for the use of animal data as an endpoint.

Postmarketing studies are critical in both of these situations to verify and describe the clinical benefit of the drug or biological product. The postmarketing studies may provide us with data that directly verify that the product provides the desired benefit in humans, such as increased survival or prevention of major morbidity.

(Comment 1) One comment suggested that we define "lethal" and "permanently disabling." The comment expressed concern that without such definitions, subpart I of part 314 and subpart H of part 601 will be misapplied in situations where clinical testing can and should be carried out.

The definitions of "lethal" and "permanently disabling" would seem to be well understood. Although we share the concern that too expansive an interpretation of "lethal" or "permanently disabling" could lead to attempts to apply this rule when human studies are, in fact, feasible, we are also concerned that too restrictive a definition of "lethal" or "permanently disabling" could lead to failure to apply subpart I of part 314 and subpart H of part 601 in situations where they should be applied to protect the public health. We believe that, as a general matter, we must rely on the good sense and responsibility of those health professionals who will be seeking to apply subpart I of part 314 and subpart H of part 601 in the future, and on responsible review of specific cases by FDA. Nevertheless, we can provide guidance for applying subpart I of part 314 and subpart H of part 601 by clarifying that a "lethal substance" is one that is likely to kill at least some of the humans who have been exposed to the substance and a "permanently disabling substance" is one that is likely to cause a permanent physical or mental impairment that substantially limits one or more of the major life activities in at least some of the humans who have been exposed to the substance.

(Comment 2) One comment stated that the rule does not explicitly cover infectious substances and pointed out that not all infectious substances produce toxins. The comment suggested replacing "toxic" with "toxic and/or infectious" in proposed §§ 314.600 and 601.60 (§ 601.90 in this final rule).

The rule is certainly intended to cover products for treatment of infections. At some level, an infectious agent that is lethal or permanently disabling is toxic to its host, even if that agent is not itself a "toxin" or a producer of "toxins" within a strict definition of the word. Because we do not use "toxin" in the rule, and "toxic" is accurate, we do not believe we need to replace "toxic" with "toxic and/or infectious" to indicate that products for the treatment of infections may be approved under this rule.

(Comment 3) One comment noted that the proposed rule did not discuss criteria that should be applied in determining if "an important medical need is not adequately met by currently available therapies." The comment suggested that we state that we will use the criteria given in our guidance for industry entitled "Fast Track Drug Development Programs—Designation, Development, and Application Review" (September 1998).

We have decided to eliminate the requirement that "products would be expected to provide meaningful therapeutic benefits to patients over existing treatments," as well as the limitation that the toxic agent be "without a proven treatment" (proposed §§ 314.600 and 601.60). Recent events involving the multiple exposures to anthrax in our population, and deaths resulting from those infections, have indicated a need for a wide range of therapeutic options that, in some instances, might be inappropriately limited by requiring new products to have a therapeutic benefit over existing treatments, or to be used only in the absence of a proven treatment. Availability of a variety of drug and biological products is important because, for example, patient recipients may be allergic to one product and require another, may be intolerant of a product because of side effects, or may respond more favorably to one product

[2] In some cases, however, such as with anti-infective drug products, it would usually be expected that human data on safety and effectiveness for other indications may be available.

than another. We also believe that a wider variety of therapeutic choices will limit potential problems with availability, accessibility, and distribution of products. We have modified the final rule to address these concerns and help ensure the availability of more than one therapeutic option.

(Comment 4) One comment requested that antivenin and antitoxin products of animal origin be considered for inclusion specifically on the list of new drugs and biological products to which the rule applies.

There is no list of products that may be approved based on evidence of effectiveness from efficacy studies in animals. The rule provides criteria to determine if evidence of effectiveness from efficacy studies in animals may support approval of a product. If an antivenin or antitoxin product of animal origin meets the criteria specified in the rule, it may be approved on the basis of evidence of effectiveness from efficacy studies in animals.

(Comment 5) One comment requested that we revise proposed §§ 314.610 and 601.61 (§ 601.91 in this final rule) to state that substantiation in multiple animal species is required only where appropriate. The comment stated we should not limit ourselves to approvals only when there is substantiation in "multiple" animal species. The comment contended that where independent studies in a single species meet the general principles of independent substantiation as described in the guidance for industry entitled "Providing Clinical Evidence of Effectiveness for Human Drugs and Biological Products" (May 1998), those studies are sufficient to substantiate effectiveness as a matter of science and a requirement of substantiation in multiple species would result in an unnecessary delay of agency approval. According to the comment, these concerns are particularly important where viruses have a narrow host range and conducting efficacy trials in more than one animal species in such cases either is not feasible or provides only limited additional information that is relevant to the full-blown disease in humans. The comment suggested that the requirement of substantiation in multiple species in a given case should depend on the known host range and the availability of animal model systems.

We share some of the concerns expressed in the comment, but we believe the proposed remedy goes too far. Approval of the use of a drug lacking human evidence of effectiveness represents a significant departure from ordinary practice. There are countless examples of treatments with favorable effects in animals that did not prove effective in humans. Although this rule does, for good reason, allow reliance on animal studies when human studies cannot be conducted, in general we expect that the evidence, to be persuasive, should be developed in more than one animal species unless the effect is demonstrated in a single animal species that represents a sufficiently well-characterized animal model for predicting the response in humans. We recognize that conducting studies in more than one species can result in added expense, but we believe this is warranted because of the additional assurance they would provide.

Furthermore, reliance on our guidance entitled "Providing Clinical Evidence of Effectiveness for Human Drugs and Biological Products" is misplaced. That guidance was drafted to provide advice on the quantity of data from clinical studies needed to support a finding of effectiveness and, specifically, on when the agency ought to rely on a single human study. The guidance addressed cases in which the issue is the credibility of the data itself, not the relevance of the data to humans. In this rule, the issue is the ability of results from animal studies to predict the human response, and not the credibility of the animal finding itself (although, of course, the animal studies should be replicated or substantiated in each species as needed to ensure credible results). The need for multiple species in certain cases is to enhance the likelihood that the data are pertinent to humans.

We do recognize, however, that the multiple species requirement could be inappropriate or unnecessary in certain situations. For example, there may be only one species capable of reacting with a response predictive for humans. This would occur where there is only one nonhuman host for the targeted microorganism. There may also be other situations in which studies in a particular species are specifically well recognized as predictors of effectiveness in humans. Thus, circumstances in which the agency will rely on evidence from studies in one animal species to provide substantial evidence of the effectiveness of these products in humans would generally be limited to situations where the study model is sufficiently well-recognized so as to render studies in multiple species unnecessary. In addition, other human data for the product could provide support for such approvals.

Accordingly, we have changed proposed §§ 314.610 and 601.61 (§ 601.91(c) in this final rule) to require that approval be based on studies in more than one animal species unless the effect is demonstrated in a single animal species that represents a sufficiently well-characterized animal model for predicting the response in humans. The agency believes that demonstrating effectiveness in studies conducted in a single animal species using a well-characterized animal model will most often be done for anti-infective drug products. The pathophysiological mechanisms of infectious diseases are usually very well understood, and animal models for many infectious diseases have been studied for years and are very well characterized.

(Comment 6) One comment suggested we remove the requirement that there be a reasonably well-understood pathophysiological mechanism of the toxicity of the substance and its prevention or substantial reduction by the product. The comment stated it is hard to say when we understand something reasonably well and that, if we decide to retain the requirement, we should state at what level (e.g., cellular, molecular) the mechanism must be understood.

A disease's or toxin's mechanism of action does not need to be understood before a safe and effective treatment or preventative can be devised. Quinine and Jenner's smallpox vaccine were both developed before the acceptance of the germ theory of disease. Neither is there a general requirement that an applicant who is relying on human testing to establish effectiveness demonstrate the mechanism of action of the drug or biological product that is the subject of the marketing application. It is generally sufficient to demonstrate that a product is safe and effective. It is generally not required that an applicant demonstrate how or why the product is safe and effective.

It is true that a pathophysiologic understanding of a disease and treatment is not required when human studies are used to support approval. In the case of human drug or biological products approved on the basis of evidence of effectiveness from studies in animals, however, we are requiring an understanding of the mechanism of the toxic substance or infectious organism and its prevention or reduction by the product. This understanding helps provide assurance that the efficacy data from studies in animals can be applied to humans. We have not specified exactly what degree of pathophysiologic understanding is needed, and that will be a matter of judgment. The level of understanding could range from a complete understanding of how a toxic

substance works at the cellular level in both human and animal cells together with a clear understanding of what the antidote does at the molecular level to a less complete understanding. The level of required understanding of the mechanism of action of the toxic substance or infectious organism and the product may vary from toxic substance to toxic substance or infectious organism to infectious organism and could even vary from one product to another intended to treat the same condition.

(Comment 7) One comment suggested that an institutional review board (IRB) or other ethical scientific review body determine if it would be unethical to conduct studies in humans. The comment also said we do not mention who would make the determination that it would be unethical to conduct studies in humans.

The final determination that it is unethical to conduct studies in humans will be made by the reviewing officials in FDA. We anticipate that in most cases the determination as to whether it would be unethical to conduct studies in humans will not be difficult. In those cases that are difficult, the views of one or more IRBs, individual ethicists and clinicians, and FDA advisory committees could be sought by a sponsor or FDA. A case where such a consultation could be useful is one in which a putatively subtoxic dose would be used in humans to establish at least a mechanism for protection, if not actual protection.

(Comment 8) One comment noted that we said in the proposed rule:

> The agency also intends in most cases to consult on applications to market such products with an advisory committee, supplemented with appropriate expert consultants, in meetings open to the public in order to receive expert advice on whether a particular set of animal data support efficacy of a product under this rule (64 FR 53960 at 53964 and 53965).

The comment asked us to consider requiring consultation with an advisory committee either before conducting the animal studies or before approval of the product, or both.

We want to reiterate our statement in the proposed rule that we intend usually to consult with an advisory committee during the approval process. Indeed, we may consult with an advisory committee more than once on a single product if circumstances warrant it. Consultation with an advisory committee could occur early in the development process, to discuss whether the concept of using certain animal data to support efficacy is reasonable.

Even though consultation with an advisory committee is generally desirable, it is not always practical. For example, products reviewed under this rule may be part of the response to a public health emergency; therefore, there may not be time to convene an advisory committee. Accordingly, we believe that it would be inappropriate to absolutely require consultation with an advisory committee.

(Comment 9) One comment questioned whether patient labeling is adequate to inform patients that a product has been approved on the basis of animal efficacy data, particularly in situations where military personnel are ordered to take a product approved under this rule. The comment did not suggest an alternative to the provisions of the rule.

Sections 314.610(b)(3) and 609.91(b)(3) provide that for products or specific indications approved under this rule, applicants must prepare, as part of their proposed labeling, labeling to be provided to patients or potential patients. The patient labeling, written in language that can be easily understood by the general public, must explain that, for ethical or feasibility reasons, the product's approval was based on efficacy studies conducted in animals alone. The labeling must give the product's indication(s), directions for use (dosage and administration), contraindications, a description of any reasonably foreseeable risks, adverse reactions, anticipated benefits, drug interactions, and any other relevant information required by FDA at the time of approval. If possible, the patient labeling must be available with the product to be provided to patients or potential patients prior to administration or dispensing of the product for the use approved under this rule. We intend that in interpreting §§ 314.610(b)(3) and 601.91(b)(3), the word "possible" be given its ordinary and literal meaning. Situations in which it would be inconvenient or require some effort to make the labeling available for patients should not be equated with situations in which it would be impossible to do so.

These provisions, coupled with communications within a health care provider-patient relationship should, as a general matter in both civilian and military contexts, adequately ensure that patients are informed that the product they are taking has been approved based on animal efficacy data.

(Comment 10) One comment suggested that labeling a drug or biological product approved on the basis of evidence of effectiveness from studies in animals as "FDA approved" is misleading, because patients would assume that the product had been approved based on human studies. The comment suggested that we treat the product as an investigational new drug, but waive certain requirements generally applied to investigational new drugs, if those requirements would provide obstacles to the product's use in an emergency.

We agree that the labeling would be misleading if information were not included to explain to patients or potential patients that the effectiveness of the product was demonstrated in animals not humans, and that this reliance on animal efficacy data was based on ethical and feasibility concerns. Therefore, under sections 502(a) and 701(a) of the Federal Food, Drug, and Cosmetic Act (the act) (21 U.S.C. 352(a) and 372(a)) (and consistent with the legal authority cited in the preamble to the proposed rule (64 FR 53960 at 53964)), we have revised the language in §§ 314.610(b)(3) and 601.91(b)(3) to require that this information be included in the patient labeling.

Where the evidence of effectiveness comes from studies in animals, regulating new drug or biological products as investigational drugs presents several difficulties. These difficulties have led us to this rulemaking. The proposed rule describes our concerns with relying solely on the investigational new drug regulations (64 FR 53960 at 53963) for such approvals. There may be cases, however, when an application does not meet the criteria of this rule, and approval of the product is not feasible. Should an emergency situation arise under such circumstances, it is conceivable that the product could be used under the investigational new drug regulations.

(Comment 11) Another comment suggested that, unless "lay persons" may use the product, we prohibit advertising of drug or biological products approved on the basis of evidence of effectiveness from studies in animals. The comment further recommended stringent controls on the advertising of products that could be used by "lay persons."

Such a sweeping prohibition would likely give rise to constitutional issues regarding the regulation of commercial speech. In addition, the suggestion presents serious public health concerns. A prohibition on advertising could limit health care providers' and public health and emergency preparedness officials' awareness of the products approved under this rule. Limiting awareness of these products, which are intended to

reduce or prevent life-threatening or disabling toxicity, does not seem desirable or appropriate.

We believe that the advertising provisions in §§ 314.640 and 601.94 of this rule provide adequate protection against false or misleading advertising, and no additional requirements are needed. As discussed in the preamble to the proposed rule (64 FR 53960 at 53964), we proposed the requirements pertaining to promotional materials in order to provide for the safe and effective use of these products. These requirements, along with others, are similar to those in the accelerated approval regulations in subpart H of part 314 and in subpart E of part 601. In issuing the accelerated approval regulations, we stated that the special circumstances under which those products would be approved and the possibility that promotional materials could adversely affect the sensitive risk/benefit balance justified review of promotional materials before and after approval (57 FR 58942 at 58949). Similarly, the special circumstances of all product approvals under subpart I of part 314 and subpart H of part 601 and the possibility that promotional materials could adversely affect the even more sensitive risk/benefit balance justifies advance review of promotional materials.

We intend to review all such promotional materials under these new regulations promptly, and to notify the applicant of any identified problems as soon as possible (see also 57 FR 58942 at 58950). Also as with the accelerated approval regulations' requirements for promotional materials (§§ 314.560 and 601.46), FDA may terminate the requirements for advance submission of promotional materials under these new regulations at §§ 314.650 and 601.95 if the agency determines, on its own initiative or in response to a petition submitted by the sponsor, that the requirements are no longer necessary for safe and effective use of the product. When we remove the requirement for advance submission of promotional materials, we will continue to offer a prompt review of all voluntarily submitted promotional materials.

(Comment 12) We received some comments addressing questions posed in section VII, "Discussion," of the proposed rule. In this final rule, we have addressed comments that dealt with the rule itself. Comments that dealt with questions related to the application of this rule, rather than the requirements, will be addressed if and when we draft a guidance on this subject.

III. Legal Authority

We did not receive any comments discussing our legal authority to approve new drugs and biological products based on evidence of effectiveness from studies in animals. We have concluded, for the reasons set out in section V of the proposed rule, "Legal Authority," (64 FR 53960 at 53964), that we have the legal authority to approve new drugs and biological products based on evidence of effectiveness from studies in animals.

(Comment 13) We received a comment asserting that under the court's holding in *American Pharmaceutical Association* v. *Weinberger*, 377 F.Supp. 824 (D.C.D.C. 1974) *aff'd sub nom. American Pharmaceutical Association* v. *Mathews*, 530 F.2d 1054 (D.C. Cir. 1976) (*per curiam*), we do not have the legal authority to impose the distribution controls proposed in §§ 314.610(b) and 601.61(b) (§§ 314.610(b)(2) and 601.91(b)(2) in this final rule). The comment asked that, if we disagree with their characterization of the law, distribution controls not be applied just because a product was approved under the provisions of this rule. The comment also asked that we give examples of situations where we would impose distribution restrictions.

For a full discussion of FDA's authority to impose distribution restrictions to ensure the safe use of drug products, see the agency's proposed and final rules amending part 314 by adding subpart H on accelerated approval of new drugs for serious or life-threatening illnesses (proposed rule at 57 FR 13234, April 15, 1992; final rule at 57 FR 59842, December 11, 1992). Those rules relied on sections 501, 502, 503, 505, and 701 of the act (21 U.S.C. 351, 352, 353, 355, and 372) as authority for FDA to issue regulations to help ensure the safety and effectiveness of new drugs.

We agree with the comment that distribution controls should not be placed on a product solely because it is approved under the provisions of this rule. New §§ 314.610(b)(2) and 601.91(b)(2) authorize distribution controls—they do not require them.

We do not believe it would be useful to give examples of situations where distribution controls may be necessary to ensure safe use of the product. Products approved under this rule could be indicated for widely differing conditions, and those products could be used in unique circumstances presenting many distinct safety concerns. It would not be practical to try to devise a list of representative examples of situations where distribution controls would be appropriate.

IV. Environmental Impact

The agency has determined under 21 CFR 25.30(h) that this action is of a type that does not individually or cumulatively have a significant effect on the human environment. Therefore, neither an environmental assessment nor an environmental impact statement is required.

V. Federalism

FDA has analyzed this final rule in accordance with the principles set forth in Executive Order 13132. FDA has determined that the rule does not contain policies that have substantial direct effects on the States, on the relationship between the National Government and the States, or on the distribution of power and responsibilities among the various levels of government. Accordingly, the agency has concluded that the rule does not contain policies that have federalism implications as defined in the Executive order and, consequently, a federalism summary impact statement is not required.

VI. Analysis of Impacts

FDA has examined the impacts of the final rule under Executive Order 12866 and the Regulatory Flexibility Act (5 U.S.C. 601–612) (as amended by subtitle D of the Small Business Regulatory Fairness Act of 1996 (Public Law 104–121)) and the Unfunded Mandates Reform Act of 1995 (Public Law 104–4). Executive Order 12866 directs agencies to assess all costs and benefits of available regulatory alternatives and, when regulation is necessary, to select regulatory approaches that maximize net benefits (including potential economic, environmental, public health and safety, and other advantages; distributive impacts; and equity). Unless the agency certifies that the rule is not expected to have a significant economic impact on a substantial number of small entities, the Regulatory Flexibility Act requires agencies to analyze regulatory options that would minimize any significant economic impact of a rule on small entities. Section 202 of the Unfunded Mandates Reform Act (Public Law 104–4) requires that agencies prepare an assessment of anticipated costs and benefits before proposing any rule that may result in expenditure by State, local, and tribal governments, in the aggregate, or by the private sector, of $100 million in any one year (adjusted annually for inflation).

The agency has determined that the rule is consistent with the principles set forth in the Executive order and in these statutes. FDA finds that this rule will not have an effect on the economy that exceeds $100 million in any one year (adjusted for inflation). The current inflation-adjusted statutory threshold is about $110 million. Therefore, no further analysis is required under the Unfunded Mandates Reform Act. Because this rule does not impose any new costs on small entities, FDA certifies that this rule will not result in a significant economic impact on a substantial number of small entities. Thus, the agency need not prepare a Regulatory Flexibility Analysis. The agency reached the same conclusions in its proposed rule. FDA has not received any new information or comments that would alter its previous determinations.

VII. The Paperwork Reduction Act of 1995

This final rule contains information collection provisions that are subject to review by the Office of Management and Budget (OMB) under the Paperwork Reduction Act of 1995 (44 U.S.C. 3501–3520). The title, description, and respondent description of the information collection provisions are shown below with an estimate of the annual reporting and recordkeeping burden. Included in the estimate is the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing each collection of information.

Title: New Drug and Biological Products; Animal Efficacy Studies.

Description: FDA is amending its new drug and biological product regulations to allow appropriate studies in animals in certain cases to provide substantial evidence of effectiveness of new drug and biological products used to reduce or prevent the toxicity of chemical, biological, radiological, or nuclear substances when adequate and well-controlled efficacy studies in humans cannot be ethically conducted because the studies would involve administering a potentially lethal or permanently disabling toxic substance or organism to healthy human volunteers and field trials are not feasible prior to approval. In these circumstances, when it may be impossible to demonstrate effectiveness through adequate and well-controlled studies in humans, FDA is providing that certain new drug and biological products intended to treat or prevent serious or life-threatening conditions could be approved for marketing based on studies in animals, without the traditional efficacy studies in humans. FDA is taking this action because it recognizes the importance of improving medical response capabilities to the use of lethal or permanently disabling chemical, biological, radiological, and nuclear substances in order to protect individuals exposed to these substances.

Respondent Description: Businesses and other for-profit organizations, and nonprofit institutions.

TABLE 1.—ESTIMATED ANNUAL REPORTING BURDEN[1]

21 CFR Section	No. of Respondents	Annual Frequency per Response	Total Annual Responses	Hours per Response	Total Hours
314.610(b)(2) and 314.630 601.91(b)(2) and 601.93	1	1	1	5	5
314.610(b) and 314.640 601.91(b) and 601.94	1	1	1	240	240
Total					245

[1] There are no capital costs or operating and maintenance costs associated with this collection of information.

TABLE 2.—ESTIMATED ANNUAL DISCLOSURE/RECORDKEEPING BURDEN[1]

21 CFR Section	No. of Record-keepers	Annual Frequency per Recordkeeping	Total Annual Records	Hours per Recordkeeper	Total Hours
314.610(b)(2) and 314.630 601.91(b)(2) and 601.93	1	1	1	1	1
314.610(b) 601.91(b)	1	1	1	1	1
Total					2

[1]There are no capital costs or operating and maintenance costs with this collection of information.

FDA estimates that only one application of this nature may be submitted every 3 years; however, for calculation purposes, FDA is estimating the submission of one application annually. FDA estimates 240 hours for a manufacturer of a new drug or biological product to develop patient labeling and to submit the appropriate information and promotional labeling to FDA. At this time, FDA cannot estimate the number of postmarketing reports for adverse drug or biological experiences associated with a newly approved drug or biological product. Therefore, FDA is using one report for purposes of this information collection. These reports are required under parts 310 and 600 (21 CFR parts 310 and 600), and 314. Any burdens associated with these requirements will be reported under the adverse experience reporting (AER) information collection requirements. The estimated hours for postmarketing reports range from 1 to 5 hours based on previous estimates for AER; however FDA is estimating 5 hours for the purpose of this information collection.

The majority of the burden for developing the patient labeling is included under the reporting requirements; therefore, minimal burden is calculated for providing the guide to patients. As discussed previously, no burden can be calculated at this time for the number of AER reports that may be submitted after approval of a new drug or biologic. Therefore, the number of records that may be maintained also cannot be determined. Any burdens associated with these requirements will be reported under the AER information collection requirements. The estimated recordkeeping burden of 1 hour is based on previous estimates for the recordkeeping requirements associated with the AER system.

The information collection provisions in this final rule have been approved under OMB control number 0910–0423. This approval expires December 31, 2002. An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB control number.

List of Subjects

21 CFR Part 314

Administrative practice and procedure, Confidential business information, Drugs, Reporting and recordkeeping requirements.

21 CFR Part 601

Administrative practice and procedure, Biologics, Confidential business information.

Therefore, under the Federal Food, Drug, and Cosmetic Act and under authority delegated to the Commissioner of Food and Drugs, 21 CFR parts 314 and 601 are amended as follows:

PART 314—APPLICATIONS FOR FDA APPROVAL TO MARKET A NEW DRUG

1. The authority citation for 21 CFR part 314 continues to read as follows:

Authority: 21 U.S.C. 321, 331, 351, 352, 353, 355, 355a, 356, 356a, 356b, 356c, 371, 374, 379e.

2. Subpart I, consisting of §§ 314.600 through 314.650, is added to read as follows:

Subpart I—Approval of New Drugs When Human Efficacy Studies Are Not Ethical or Feasible

Sec.

Subpart I—Approval of New Drugs When Human Efficacy Studies Are Not Ethical or Feasible

§ 314.600 Scope.

This subpart applies to certain new drug products that have been studied for their safety and efficacy in ameliorating or preventing serious or life-threatening conditions caused by exposure to lethal or permanently disabling toxic biological, chemical, radiological, or nuclear substances. This subpart applies only to those new drug products for which: Definitive human efficacy studies cannot be conducted because it would be unethical to deliberately expose healthy human volunteers to a lethal or permanently disabling toxic biological, chemical, radiological, or nuclear substance; and field trials to study the product's effectiveness after an accidental or hostile exposure have not been feasible. This subpart does not apply to products that can be approved based on efficacy standards described elsewhere in FDA's regulations (e.g., accelerated approval based on surrogate markers or clinical endpoints other than survival or irreversible morbidity), nor does it address the safety evaluation for the products to which it does apply.

§ 314.610 Approval based on evidence of effectiveness from studies in animals.

(a) FDA may grant marketing approval for a new drug product for which safety has been established and for which the requirements of § 314.600 are met based on adequate and well-controlled animal studies when the results of those animal studies establish that the drug product is reasonably likely to produce clinical benefit in humans. In assessing the sufficiency of animal data, the agency may take into account other data, including human data, available to the agency. FDA will rely on the evidence from studies in animals to provide substantial evidence of the effectiveness of these products only when:

(1) There is a reasonably well-understood pathophysiological mechanism of the toxicity of the substance and its prevention or substantial reduction by the product;

(2) The effect is demonstrated in more than one animal species expected to react with a response predictive for humans, unless the effect is demonstrated in a single animal species that represents a sufficiently well-characterized animal model for predicting the response in humans;

(3) The animal study endpoint is clearly related to the desired benefit in humans, generally the enhancement of survival or prevention of major morbidity; and

(4) The data or information on the kinetics and pharmacodynamics of the product or other relevant data or information, in animals and humans, allows selection of an effective dose in humans.

(b) Approval under this subpart will be subject to three requirements:

(1) *Postmarketing studies.* The applicant must conduct postmarketing studies, such as field studies, to verify and describe the drug's clinical benefit and to assess its safety when used as indicated when such studies are feasible and ethical. Such postmarketing studies would not be feasible until an exigency arises. When such studies are feasible, the applicant must conduct such studies with due diligence. Applicants must include as part of their application a plan or approach to postmarketing study commitments in the event such studies become ethical and feasible.

(2) *Approval with restrictions to ensure safe use.* If FDA concludes that a drug product shown to be effective under this subpart can be safely used only if distribution or use is restricted, FDA will require such postmarketing restrictions as are needed to ensure safe use of the drug product, commensurate with the specific safety concerns presented by the drug product, such as:

(i) Distribution restricted to certain facilities or health care practitioners with special training or experience;

(ii) Distribution conditioned on the performance of specified medical procedures, including medical followup; and

(iii) Distribution conditioned on specified recordkeeping requirements.

(3) *Information to be provided to patient recipients.* For drug products or specific indications approved under this subpart, applicants must prepare, as part of their proposed labeling, labeling to be provided to patient recipients. The patient labeling must explain that, for ethical or feasibility reasons, the drug's approval was based on efficacy studies conducted in animals alone and must give the drug's indication(s), directions for use (dosage and administration), contraindications, a description of any reasonably foreseeable risks, adverse reactions, anticipated benefits, drug interactions, and any other relevant information required by FDA at the time of approval. The patient labeling must be available with the product to be provided to patients prior to administration or dispensing of the drug product for the use approved under this subpart, if possible.

§ 314.620 Withdrawal procedures.

(a) *Reasons to withdraw approval.* For new drugs approved under this subpart, FDA may withdraw approval, following a hearing as provided in part 15 of this chapter, as modified by this section, if:

(1) A postmarketing clinical study fails to verify clinical benefit;

(2) The applicant fails to perform the postmarketing study with due diligence;

(3) Use after marketing demonstrates that postmarketing restrictions are inadequate to ensure safe use of the drug product;

(4) The applicant fails to adhere to the postmarketing restrictions applied at the time of approval under this subpart;

(5) The promotional materials are false or misleading; or

(6) Other evidence demonstrates that the drug product is not shown to be safe or effective under its conditions of use.

(b) *Notice of opportunity for a hearing.* The Director of the Center for Drug Evaluation and Research (CDER) will give the applicant notice of an opportunity for a hearing on CDER's proposal to withdraw the approval of an application approved under this subpart. The notice, which will ordinarily be a letter, will state generally the reasons for the action and the proposed grounds for the order.

(c) *Submission of data and information.* (1) If the applicant fails to file a written request for a hearing within 15 days of receipt of the notice, the applicant waives the opportunity for a hearing.

(2) If the applicant files a timely request for a hearing, the agency will publish a notice of hearing in the **Federal Register** in accordance with §§ 12.32(e) and 15.20 of this chapter.

(3) An applicant who requests a hearing under this section must, within 30 days of receipt of the notice of opportunity for a hearing, submit the data and information upon which the applicant intends to rely at the hearing.

(d) *Separation of functions.* Separation of functions (as specified in § 10.55 of this chapter) will not apply at any point in withdrawal proceedings under this section.

(e) *Procedures for hearings.* Hearings held under this section will be conducted in accordance with the provisions of part 15 of this chapter, with the following modifications:

(1) An advisory committee duly constituted under part 14 of this chapter will be present at the hearing. The committee will be asked to review the issues involved and to provide advice and recommendations to the Commissioner of Food and Drugs.

(2) The presiding officer, the advisory committee members, up to three representatives of the applicant, and up to three representatives of CDER may question any person during or at the conclusion of the person's presentation. No other person attending the hearing may question a person making a presentation. The presiding officer may, as a matter of discretion, permit questions to be submitted to the presiding officer for response by a person making a presentation.

(f) *Judicial review.* The Commissioner of Food and Drugs' decision constitutes final agency action from which the applicant may petition for judicial review. Before requesting an order from a court for a stay of action pending review, an applicant must first submit a petition for a stay of action under § 10.35 of this chapter.

§ 314.630 Postmarketing safety reporting.

Drug products approved under this subpart are subject to the postmarketing recordkeeping and safety reporting requirements applicable to all approved drug products, as provided in §§ 314.80 and 314.81.

§ 314.640 Promotional materials.

For drug products being considered for approval under this subpart, unless otherwise informed by the agency, applicants must submit to the agency for consideration during the preapproval review period copies of all promotional materials, including promotional labeling as well as advertisements, intended for dissemination or publication within 120 days following marketing approval. After 120 days following marketing approval, unless otherwise informed by the agency, the applicant must submit promotional materials at least 30 days prior to the intended time of initial dissemination of the labeling or initial publication of the advertisement.

§ 314.650 Termination of requirements.

If FDA determines after approval under this subpart that the requirements established in §§ 314.610(b)(2), 314.620, and 314.630 are no longer necessary for the safe and effective use of a drug product, FDA will so notify the applicant. Ordinarily, for drug products approved under § 314.610, these requirements will no longer apply when FDA determines that the postmarketing study verifies and describes the drug product's clinical benefit. For drug products approved under § 314.610, the restrictions would no longer apply when FDA determines that safe use of the drug product can be ensured through appropriate labeling. FDA also retains the discretion to remove specific postapproval requirements upon review of a petition submitted by the sponsor in accordance with § 10.30 of this chapter.

PART 601—LICENSING

3. The authority citation for 21 CFR part 601 continues to read as follows:

Authority: 15 U.S.C. 1451–1561; 21 U.S.C. 321, 351, 352, 353, 355, 356b, 360, 360c–360f, 360h–360j, 371, 374, 379e, 381; 42 U.S.C. 216, 241, 262, 263, 264; sec. 122, Pub. L. 105–115, 111 Stat. 2322 (21 U.S.C. 355 note).

4. Subpart H, consisting of §§ 601.90 through 601.95, is added to read as follows:

Subpart H—Approval of Biological Products When Human Efficacy Studies Are Not Ethical or Feasible

Sec.

Subpart H—Approval of Biological Products When Human Efficacy Studies Are Not Ethical or Feasible

§ 601.90 Scope.

This subpart applies to certain biological products that have been studied for their safety and efficacy in ameliorating or preventing serious or life-threatening conditions caused by exposure to lethal or permanently disabling toxic biological, chemical, radiological, or nuclear substances. This subpart applies only to those biological products for which: Definitive human efficacy studies cannot be conducted because it would be unethical to deliberately expose healthy human volunteers to a lethal or permanently disabling toxic biological, chemical, radiological, or nuclear substance; and field trials to study the product's efficacy after an accidental or hostile exposure have not been feasible. This subpart does not apply to products that can be approved based on efficacy standards described elsewhere in FDA's regulations (e.g., accelerated approval based on surrogate markers or clinical endpoints other than survival or irreversible morbidity), nor does it address the safety evaluation for the products to which it does apply.

§ 601.91 Approval based on evidence of effectiveness from studies in animals.

(a) FDA may grant marketing approval for a biological product for which safety has been established and for which the requirements of § 601.90 are met based on adequate and well-controlled animal studies when the results of those animal studies establish that the biological product is reasonably likely to produce clinical benefit in humans. In assessing the sufficiency of animal data, the agency may take into account other data, including human data, available to the agency. FDA will rely on the evidence from studies in animals to provide substantial evidence of the effectiveness of these products only when:

(1) There is a reasonably well-understood pathophysiological mechanism of the toxicity of the

substance and its prevention or substantial reduction by the product;

(2) The effect is demonstrated in more than one animal species expected to react with a response predictive for humans, unless the effect is demonstrated in a single animal species that represents a sufficiently well-characterized animal model for predicting the response in humans;

(3) The animal study endpoint is clearly related to the desired benefit in humans, generally the enhancement of survival or prevention of major morbidity; and

(4) The data or information on the kinetics and pharmacodynamics of the product or other relevant data or information, in animals and humans, allows selection of an effective dose in humans.

(b) Approval under this subpart will be subject to three requirements:

(1) *Postmarketing studies.* The applicant must conduct postmarketing studies, such as field studies, to verify and describe the biological product's clinical benefit and to assess its safety when used as indicated when such studies are feasible and ethical. Such postmarketing studies would not be feasible until an exigency arises. When such studies are feasible, the applicant must conduct such studies with due diligence. Applicants must include as part of their application a plan or approach to postmarketing study commitments in the event such studies become ethical and feasible.

(2) *Approval with restrictions to ensure safe use.* If FDA concludes that a biological product shown to be effective under this subpart can be safely used only if distribution or use is restricted, FDA will require such postmarketing restrictions as are needed to ensure safe use of the biological product, commensurate with the specific safety concerns presented by the biological product, such as:

(i) Distribution restricted to certain facilities or health care practitioners with special training or experience;

(ii) Distribution conditioned on the performance of specified medical procedures, including medical followup; and

(iii) Distribution conditioned on specified recordkeeping requirements.

(3) *Information to be provided to patient recipients.* For biological products or specific indications approved under this subpart, applicants must prepare, as part of their proposed labeling, labeling to be provided to patient recipients. The patient labeling must explain that, for ethical or feasibility reasons, the biological product's approval was based on efficacy studies conducted in animals alone and must give the biological product's indication(s), directions for use (dosage and administration), contraindications, a description of any reasonably foreseeable risks, adverse reactions, anticipated benefits, drug interactions, and any other relevant information required by FDA at the time of approval. The patient labeling must be available with the product to be provided to patients prior to administration or dispensing of the biological product for the use approved under this subpart, if possible.

§ 601.92 Withdrawal procedures.

(a) *Reasons to withdraw approval.* For biological products approved under this subpart, FDA may withdraw approval, following a hearing as provided in part 15 of this chapter, as modified by this section, if:

(1) A postmarketing clinical study fails to verify clinical benefit;

(2) The applicant fails to perform the postmarketing study with due diligence;

(3) Use after marketing demonstrates that postmarketing restrictions are inadequate to ensure safe use of the biological product;

(4) The applicant fails to adhere to the postmarketing restrictions applied at the time of approval under this subpart;

(5) The promotional materials are false or misleading; or

(6) Other evidence demonstrates that the biological product is not shown to be safe or effective under its conditions of use.

(b) *Notice of opportunity for a hearing.* The Director of the Center for Biologics Evaluation and Research (CBER) will give the applicant notice of an opportunity for a hearing on CBER's proposal to withdraw the approval of an application approved under this subpart. The notice, which will ordinarily be a letter, will state generally the reasons for the action and the proposed grounds for the order.

(c) *Submission of data and information.* (1) If the applicant fails to file a written request for a hearing within 15 days of receipt of the notice, the applicant waives the opportunity for a hearing.

(2) If the applicant files a timely request for a hearing, the agency will publish a notice of hearing in the **Federal Register** in accordance with §§ 12.32(e) and 15.20 of this chapter.

(3) An applicant who requests a hearing under this section must, within 30 days of receipt of the notice of opportunity for a hearing, submit the data and information upon which the applicant intends to rely at the hearing.

(d) *Separation of functions.* Separation of functions (as specified in § 10.55 of this chapter) will not apply at any point in withdrawal proceedings under this section.

(e) *Procedures for hearings.* Hearings held under this section will be conducted in accordance with the provisions of part 15 of this chapter, with the following modifications:

(1) An advisory committee duly constituted under part 14 of this chapter will be present at the hearing. The committee will be asked to review the issues involved and to provide advice and recommendations to the Commissioner of Food and Drugs.

(2) The presiding officer, the advisory committee members, up to three representatives of the applicant, and up to three representatives of CBER may question any person during or at the conclusion of the person's presentation. No other person attending the hearing may question a person making a presentation. The presiding officer may, as a matter of discretion, permit questions to be submitted to the presiding officer for response by a person making a presentation.

(f) *Judicial review.* The Commissioner of Food and Drugs' decision constitutes final agency action from which the applicant may petition for judicial review. Before requesting an order from a court for a stay of action pending review, an applicant must first submit a petition for a stay of action under § 10.35 of this chapter.

§ 601.93 Postmarketing safety reporting.

Biological products approved under this subpart are subject to the postmarketing recordkeeping and safety reporting applicable to all approved biological products.

§ 601.94 Promotional materials.

For biological products being considered for approval under this subpart, unless otherwise informed by the agency, applicants must submit to the agency for consideration during the preapproval review period copies of all promotional materials, including promotional labeling as well as advertisements, intended for dissemination or publication within 120 days following marketing approval. After 120 days following marketing approval, unless otherwise informed by the agency, the applicant must submit promotional materials at least 30 days prior to the intended time of initial dissemination of the labeling or initial publication of the advertisement.

601.95 Termination of requirements.

If FDA determines after approval under this subpart that the requirements established in §§ 601.91(b)(2), 601.92, and 601.93 are no longer necessary for the safe and effective use of a biological product, FDA will so notify the applicant. Ordinarily, for biological products approved under § 601.91, these requirements will no longer apply when FDA determines that the postmarketing study verifies and describes the biological product's clinical benefit. For biological products approved under § 601.91, the restrictions would no longer apply when FDA determines that safe use of the biological product can be ensured through appropriate labeling. FDA also retains the discretion to remove specific postapproval requirements upon review of a petition submitted by the sponsor in accordance with § 10.30 of this chapter.

Dated: May 23, 2002.

Lester M. Crawford,

Deputy Commissioner.

[FR Doc. 02–13583 Filed 5–30–02; 8:45 am]

BILLING CODE 4160–01–S

DEPARTMENT OF THE TREASURY

Internal Revenue Service

26 CFR Part 1

[TD 8998]

RIN 1545–BA74

Loss Limitation Rules

AGENCY: Internal Revenue Service (IRS), Treasury.

ACTION: Temporary regulations.

SUMMARY: This document contains amendments to temporary regulations issued under sections 337(d) and 1502. The amendments clarify certain aspects of the temporary regulations relating to the deductibility of losses recognized on dispositions of subsidiary stock by members of a consolidated group. The amendments in these temporary regulations apply to corporations filing consolidated returns, both during and after the period of affiliation, and also affect purchasers of the stock of members of a consolidated group. The text of these temporary regulations also serves as the text of the proposed regulations set forth in the notice of proposed rulemaking on this subject in the Proposed Rules section in this issue of the **Federal Register**.

DATES: *Effective Date:* These regulations are effective May 31, 2002.

Applicability Date: For dates of applicability *see* § 1.337(d)–2T(g) and 1.1502–20T(i).

FOR FURTHER INFORMATION CONTACT: Sean P. Duffley (202) 622–7530 or Lola L. Johnson (202) 622–7550 (not toll-free numbers).

SUPPLEMENTARY INFORMATION:

Paperwork Reduction Act

The collection of information contained in these regulations has been previously reviewed and approved by the Office of Management and Budget under control number 1545–1774. Responses to this collection of information are voluntary. No material changes to this collection of information are made by these regulations.

An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a valid control number assigned by the Office of Management and Budget.

Books or records relating to the collection of information must be retained as long as their contents may become material in the administration of any internal revenue law. Generally, tax returns and tax return information are confidential, as required by 26 U.S.C. 6103.

Background

On March 12, 2002, the IRS and Treasury published in the **Federal Register** at 67 FR 11034 (2002–13 I.R.B. 668) temporary regulations under sections 337(d) and 1502 (the temporary regulations). The temporary regulations set forth rules that limit the deductibility of loss recognized by a consolidated group on the disposition of stock of a subsidiary member and that require certain basis reductions on the deconsolidation of stock of a subsidiary member. Section 1.1502–20T(i) of the temporary regulations provides that, in the case of a disposition or deconsolidation of a subsidiary before March 7, 2002, and for such transactions effected pursuant to a binding written contract entered into before March 7, 2002, that was in continuous effect until the disposition or deconsolidation, a consolidated group may determine the amount of allowable stock loss or basis reduction by applying § 1.1502–20 in its entirety, § 1.1502–20 without regard to the duplicated loss component of the loss disallowance rule, or § 1.337(d)-2T. For dispositions and deconsolidations that occur on or after March 7, 2002, and that are not within the scope of the binding contract rule, § 1.1502–20T(i) provides that allowable loss and basis reduction are determined under § 1.337(d)-2T, not § 1.1502–20.

Explanation of Provisions

Since the publication of the temporary regulations, several questions have been raised concerning the interpretation and application of the temporary regulations. In response to these questions, the IRS and Treasury are promulgating the regulations in this Treasury decision as temporary regulations to clarify and amend the temporary regulations as described below in this preamble. The following paragraphs describe these amendments.

Netting Rule

Commentators requested that § 1.337(d)-2T be amended to provide a netting rule similar to that set forth in § 1.1502–20(a)(4), pursuant to which gain and loss from certain dispositions of stock may be netted. This Treasury decision adds § 1.337(d)-2T(a)(4) to provide such a rule and also adds § 1.337(d)-2T(b)(4), which provides a similar netting rule for basis reductions on deconsolidations of subsidiary stock.

Time For Filing Election Described in § 1.1502–20T(i)

Section 1.1502–20T(i) currently provides that an election to determine allowable loss by applying § 1.1502–20 (without regard to the duplicated loss component of the loss disallowance rule) or § 1.337(d)–2T must be made by including a statement with or as part of the original return for the taxable year that includes the later of March 7, 2002, and the date of the disposition or deconsolidation of the stock of the subsidiary, or with or as part of an amended return filed before the date the original return for the taxable year that includes March 7, 2002, is due. Commentators noted that this provision may not permit the election to be made on an original return for the 2001 taxable year where the disposition occurs during the 2001 taxable year. The IRS and Treasury believe that it is appropriate to permit the election to be made on such a return. Therefore, this Treasury decision amends § 1.1502–20T(i) to provide that the statement may be filed with or as part of a timely filed (including any extensions) original return for any taxable year that includes any date on or before March 7, 2002. In addition, if the date of the disposition or deconsolidation of the stock of the subsidiary is after March 7, 2002, the statement may be filed with or as part of a timely filed (including any extensions) original return for the taxable year that includes such date. This latter alternative effectively permits the statement to be filed with the original return that includes the date

EXHIBIT 3

Immunogenicity and safety profiles of genetic vaccines against human Her-2/*neu* in cynomolgus monkeys

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Abstract

Her-2/*neu* is a well-characterized tumor-associated antigen, the overexpression of which in human carcinomas correlates with a poor prognosis. Here, we evaluated Her-2/*neu*-specific humoral and cellular immune responses in immunized monkeys after immunization with non-replicating adenovirus (AdHM) expressing the extracellular and transmembrane domain of human Her-2/*neu* (HM) and/or naked DNA vaccine (pHM-hGM-CSF) expressing human GM-CSF together with HM. Priming of monkeys with AdHM generated Her-2/*neu*-specific long-lasting Ab production. Furthermore, these Her-2/*neu*-specific Abs produced by AdHM immunization, some of which shared epitope specificity with Herceptin®, were able to induce Ab-dependent cellular cytotoxicity against Her-2-expressing target cells. Cellular immune responses were elicited in all monkeys immunized with Her-2/*neu*-expressing vaccine; IFN-γ was secreted when these splenocytes were restimulated with Her-2/*neu*-expressing autologous cells, and immunization with AdHM induced Her-2/*neu*-specific lympho-proliferative responses. Further, immunization with pHM-hGM-CSF prior to AdHM immunization noticeably enhanced cytotoxic T lymphocyte activity. In addition, we observed no abnormalities that would indicate that the genetic vaccines had toxic effects in the immunized monkeys. Thus, we can conclude that our genetic vaccines efficiently elicited Her-2/*neu*-specific humoral and cellular immune responses without causing severe adverse effects in non-human primates and that as such they warrant further clinical investigation.

Introduction

Her-2/*neu* is one of the tumor-associated antigens that are overexpressed in human carcinomas such as breast and ovarian cancer [1,2]. This overexpression is known to be correlated with malignancy and with a poor prognosis for patients with Her-2/*neu*-expressing tumors [3]. Previously, we have shown that immunization with genetic or cell-based vaccines targeting human Her-2/*neu* efficiently generated human Her-2/*neu*-specific immune responses in a murine model [4-6]. However, because Her-2/*neu* is a foreign antigen that is highly immunogenic in mice, the immunogenicity of the vaccine tested in a murine model may not represent its efficacy in humans. Immune responses against self-tumor antigens like Her-2/*neu* are under the control of self-tolerance mechanisms [2,7]. In this regard, we have shown that adenoviral vaccine expressing human Her-2/*neu* could elicit anti-tumor immunity against murine Her-2/*neu*-expressing tumors by breaking immunologic tolerance once the tumor-driven immunosuppressive environment was attenuated by the appropriate chemotherapy [7].

Before evaluating the anti-tumor efficacy of genetic vaccines in combination with chemotherapy in human clinical trials, we need to assess whether our genetic vaccine can elicit Her-2/*neu*-specific immune responses in humans. Because 98% of the amino acid sequences of Her-2/*neu* are shared in humans and rhesus monkeys (*Macaca mulatta*), the immunogenicity of genetic vaccines in monkeys may prove useful for predicting the potential of the vaccine in humans. Further, safety profiles developed in a simian model will provide more credible preclinical data than those developed in a murine model.

To assess the efficacy and the safety of anti-Her-2/*neu* genetic vaccines in large animals, we immunized monkeys with a DNA vaccine and/or adenovirus vaccine. We used human granulocyte-macrophage colony-stimulating factor (GM-CSF) as a genetic adjuvant for the DNA vaccine to enhance immune responses against Her-2/*neu*. On the basis of previous reports of the limited efficacy of DNA vaccines in patients [8,9], we combined adenoviral vaccine with DNA immunization to increase the immunogenicity of the DNA vaccine. The preclinical data obtained from our study suggest that Her-2/*neu* genetic vaccines might be both immunogenic and safe in humans.

Materials and Methods

Animals

Nine female cynomolgus monkeys (*Macaca fascicularis*) from Yunnan National Laboratory Primate Center (China) were bred in GLP facilities in Korea (Korea Institute of Toxicology) with the approval of the Association for Assessment and Accreditation of Laboratory Animal Care International.

All of the experiments were approved by the Institutional Animal Care and Use Committee in Korea Institute of Toxicology and all of the treatments were performed in accordance with the guidelines of the Korea Food & Drug Administration and the European Agency for the Evaluation of Medicinal Products.

For immunization, groups of monkey (3 monkeys/group) were intramuscularly (*i.m.*) injected with 2 ml of pHM-hGM-CSF (2 mg/ml) plasmid DNA and 1 ml of AdHM (1×10^{10}pfu/ml) (Supplementary Figure 1). Blood samples were taken from the cephalic vein using a CTP cell preparation tube (BD, Franklin Lakes, NJ) with sodium citrate to isolate PBMC and a vacuette (BD) with heparin to obtain sera. Monkeys were sacrificed on week 22 for the toxicity test and a part of the spleen was removed to obtain splenocytes. Splenocytes were frozen after red blood cell lysis.

Cell line

The Her-2/*neu*-expressing human breast carcinoma SK-BR-3 cell line was obtained from the

American Type Culture Collection (Manassas, VA). Her-2/*neu*-expressing transfectoma Her-2/CT26 cells were developed by transduction of CT26 cells with the cDNA-encoding human Her-2/*neu* [4].

Construction of vaccines

The plasmid pHM, which encodes the extracellular domain and transmembrane domain of Her-2/*neu* without the intracellular domain of Her-2/*neu*, was described previously [4,6]. HM-encoding recombinant adenovirus (AdHM) was constructed using the adenovirus genomic DNA-TPC cotransfection method outlined by the manufacturer (TaKaRa Bio, Japan) and described previously [10].

FACS and immunoblot

10 μg of pHM-hGM-CSF were transfected to 293T cells using CellPhect Transfection Kit (Amersham, Little Chalfont, Buckinghamshire, UK). Two days after transfection, the culture supernatant of 293T cells was obtained, filtered with 0.2 μm-membrane filter, and used for ELISA. The level of hGM-CSF was determined using a ELISA kit (Endogen, Rockford, IL) according to the manufacturer's manual. The harvested 293T cells were analyzed by FACS (BD) or immunoblot for the cell-surface expression of Her-2/*neu* as described previously [4].

To detect the expression of HM produced from AdHM, CT26 cells were transduced with AdHM at 100 MOI. Two days after transduction, the cells were harvested and the level of HM expression was analyzed using FACS or immunoblot as described above.

Sera from immunized monkeys were screened by FACS for Her-2/*neu*-specific antibodies. SK-BR-3 cells were incubated with serially diluted sera for one hour at 4°C, with free sera washed away. After washing, SK-BR-3 cells were incubated with goat anti-monkey IgG-FITC (Serotec, Oxford, UK) for 40 minutes at 4°C. After incubation, free antibodies were washed away and Her-2/*neu*-specific antibodies were measured by FACS calibur (BD). Antibody levels were determined by end point titration titer.

Antibody-dependent cell-mediated cytotoxicity (ADCC)

The SK-BR-3 cells were incubated with 200 μCi/ml of $Na[^{51}Cr]O_4$ (Dupont NEN, Boston, MA) at 37°C for 1.5 hr. At the end point of the labeling period, the labeled target cells were washed 3 times in RPMI 1640 media and plated in a 96-well round-bottom microplate at a concentration of 10^4 cells/well. Target cells were incubated for 15 minutes at 37°C in 5% CO_2 in the presence of diluted sera from immunized monkeys, and then for another 4 hours after human PBMC was added as effector (effector:target = 40:1). After incubation, the plate was centrifuged and the ^{51}Cr radioactivity in supernatants was determined in a Wallac 1470 Wizard™ automatic gamma counter. The percentage of specific lysis was calculated as [(experimental release - background release)/(maximal release - background release)] × 100. Background release was defined as ^{51}Cr release by targets in the absence of specific antibodies, and the maximal release was determined after lysing target cells with 0.5 % Triton X-100. The means of triplicate determinations are given.

Inhibition assay

A previously established sandwich-ELISA was modified for the inhibition assay [11]. In brief, multiwell plates (Nunc, Denmark) were coated with 2 μg/ml of anti-His6 Ab (Igtherapy, Korea) diluted in 50 mM $NaHCO_3$ (pH 9.6) overnight at 4 °C. After blocking and washing, 200 ng/ml of Her2/ECD (R&D systems) was added and incubated at room temperature for 4 hours. Plates were washed, and serially diluted monkey sera were added to the wells. After a 30-minute incubation, biotin-labeled Herceptin® (Roche) was added without washing to attain a final concentration of 6.25 ng/ml. Plates were incubated for 1.5 hours at room temperature. After washing, plates were incubated with Streptavidin-HRP (Sigma) for 30 minutes and developed with 100 μl of TMB (Moss Inc.). Thirty minutes later, 50 μl of 0.5N HCl was added to stop development, and optical density (OD) was detected at 450 nm.

Cytotoxic T cell assay

Splenocytes from immunized monkeys were re-stimulated with Mitomycin C (MMC; Sigma)–treated BLCL-HM at an effector:stimulator ratio of 20:1, as described previously [4]. Human recombinant IL-2 (rIL-2; Sigma) (1 unit/ml) was added on day 8. On day 10, cells were harvested and cytolytic activity against target cells (BLCL or BLCL-HM) was measured using a standard Cr-51 release assay. The percentage of lysis was calculated as [(sample lysis – spontaneous lysis) / (maximal lysis –

spontaneous lysis)] x 100.

Proliferation assay

Splenocytes from immunized monkeys were plated (1×10^5 spleen cells per well) in 96-well round-bottom microtiter plates (Nunc) and cultured for 4 days with the indicated numbers of MMC-treated B Lymphoblastoid Cell Lines (BLCL) or BLCL-HM. After 96 hours of incubation, including a final 24-hour pulse with [^{3}H]thymidine (1 μCi per well), the cultured splenocytes were harvested using an automatic harvester (Skatron, Sterling, VA) and [^{3}H]thymidine incorporation was measured using a liquid scintillation counter (Wallac, Turku, Finland).

IFN-γassay

Splenocytes from immunized monkeys were re-stimulated as indicated in the CTL assay. On day 10, culture supernatants were tested for the presence of IFN-γ by using a monkey IFN-γ ELISA set (BD).

Construction of BLCL and BLCL-HM

BLCL was established by infecting PBMC with *Herpesvirus papio*, which originated from S594 cell-line. It was infected with a retrovirus expressing a truncated form of Her-2/*neu* to obtain Her-2/*neu*-expressing BLCL (BLCL-HM). The expression of Her-2/*neu* on the BLCL was measured by FACS analysis.

Results

Construction of genetic vaccines expressing human Her-2/neu

We previously reported that immunization of mice with pHM-GM-CSF that expressed truncated hHer-2/*neu* and murine GM-CSF effectively generated protective and therapeutic anti-tumor immunity by inducing Her-2/*neu*-specific antibodies and cytotoxic T cells [4]. We constructed a plasmid DNA, pCK-HM-IRES-hGM-CSF (pHM-hGM-CSF), in which a truncated form of human Her-2 and human GM-CSF had been encoded independently, for the application of genetic adjuvant GM-CSF in the preclinical study using nonhuman primates.

Although the DNA vaccine has been thought to offer considerable promise in anticancer immunotherapy, the outcomes of recent clinical studies fell short of expectations [8,9]. Thus, to boost DNA-primed Her-2/*neu*-specific immune responses, we also constructed an adenoviral vaccine expressing the extracellular and transmembrane domain of human Her-2/*neu* using non-replicating adenovirus vector. The expression of Her-2/*neu* and/or hGM-CSF by the genetic vaccine constructs were confirmed by FACS and immunoblot analysis after *in vitro* transducing of CT26 or 293T cells with pHM-hGM-CSF or AdHM (Figure 1).

Immunization schema of cynomolgus monkeys

Nine monkeys were randomly divided into three groups (3 monkeys per group). Monkeys in group 1 were injected with saline instead of pHM-hGM-CSF or AdHM immunization as delineated in Supplementary Figure 1. Monkeys in group 2 were primed with AdHM alone on week 3 and boosted with three weekly *i.m.* injections of pHM-hGM-CSF on weeks 14, 15 and 16. Finally, monkeys in group 3 were primed with three weekly *i.m.* injections (4 mg/vaccination) of pHM-hGM-CSF on weeks 0, 1 and 2, followed by a single *i.m.* injection of AdHM (10^{10} pfu/vaccination) on week 3. These three monkeys, like those in group 2, were boosted on weeks 14, 15, and 16 with pHM-hGM-CSF.

Genetic immunization of monkeys generated Her-2/neu-specific humoral immune responses

To determine whether the immunization of cynomolgus monkeys with the constructed vaccines could establish humoral immune responses specific to Her-2/*neu*, we measured the titers of Her-2/*neu*-specific antibodies in sera from immunized monkeys using a SK-BR-3 cell–binding assay. The titers of sera from immunized monkeys were determined by a 2-fold increase in mean fluorescence intensity (MFI) of SK-BR-3 binding over that of respective pre-immune sera (Figure 2A). When tested with sera from the monkeys in group 2, AdHM immunization increased the titers of sera from immunized monkeys to 12,800 within 4 weeks. Although we could not detect Her-2-specific binding in sera from monkeys immunized with pHM-hGM-CSF until week 3, a boosting immunization of DNA-

primed monkeys with AdHM more rapidly increased the titers than did AdHM alone (100±50 vs 700±458). However, we could not detect any further increase in Her-2-specific Ab titers when the monkeys in groups 2 and 3 were boosted with pHM-hGM-CSF on weeks 14, 15, and 16. The overall Ab production generated by the genetic vaccine persisted at least 18 weeks. In contrast, we could not observe any Her-2/*neu*-specific binding in sera from saline-treated monkeys in group 1.

We next tested for antibodies that could share the epitope specificity with Herceptin®, the widely used monoclonal antibody to treat Her-2/*neu*-positive human carcinoma. When the sera from the immunized monkeys were tested in sandwich ELISA using Her2/ECD fusion protein, Herceptin®-binding was specifically inhibited by the sera from monkeys immunized with Her-2-expressing genetic vaccines (Figure 2B).

Because the anti-tumor effect of several antibodies was partially dependent on antibody-dependent cell-mediated cytotoxicity (ADCC) as well as direct binding with tumor antigen on the cell surface, we tested antibody-dependent killing of SK-BR-3 cells of sera using human PBMC as effectors. Immunization of monkeys with either AdHM alone or AdHM with pHM-hGM-CSF could induce antibodies capable of participating in ADCC; no significant difference was observed between group 2 and group 3 (Figure 2C). However, sera from saline-injected monkeys did not induce specific lysis of target cells.

These data show that our genetic vaccines can efficiently induce Her-2-specific humoral immune responses persisting at least 18 weeks and ADCC.

Construction of B Lymphoblastoid Cell Lines (BLCL) and BLCL-HM

Because stably transduced BLCLs expressing specific tumor antigen are useful antigen-presenting cells (APCs) that can efficiently stimulate CD4+ and CD8+ T cells as well as act as CTL targets, we generated simian BLCL by infecting PBMC with *H. papio*. BLCL-HM that stably expressed Her-2/*neu* on the cell surface was constructed by transfecting BLCL with retrovirus-expressing human Her-2/*neu*. The expression of CD20 and MHC class I on the BLCL and BLCL-HM was assessed to confirm the phenotype of BLCL (Supplementary Figure 2). Although the expression level of Her-2/*neu* differed slightly among monkeys, BLCL-HM was found to stably express significant levels of Her-2/*neu* on the cell surface (Supplementary Figure 2).

Her-2-specific lympho-proliferative capacity of splenocytes was significantly increased by genetic immunization

To test whether the splenocytes obtained from immunized monkeys were responsive against Her-2/*neu*-specific restimulation, we co-incubated splenocytes from groups of monkeys with the respective MMC-treated BLCL or BLCL-HM for 96 hours, including a final 24-hour pulse with [^{3}H]thymidine. Proliferation in BLCL-HM–stimulated splenocytes was twice that observed with BLCL stimulation in all three monkeys in group 2 (Figure 3). Although one of the three monkeys in group 3 (Monkey #7) showed a profound increase in Her-2-specific proliferation in splenocytes after stimulation with

BLCL-HM vs BLCL, the same level of proliferation was attained in the other two monkeys (Monkey #8 and #9) by BLCL stimulation alone. However, we could not detect significant levels of Her-2-specific proliferation in any of the saline-treated monkeys in group 1. These data show that our genetic vaccines elicited priming of Her-2-specific T cells in the spleen of immunized monkeys. These Her-2-specific cells in the spleen, which can function after recognition of antigen in vitro, persisted for at least 10 weeks after the final immunization.

Her-2-specific IFN-γ secretion occurred during in vitro culture of splenocytes from immunized monkeys

We tested whether the in vitro incubation of splenocytes from immunized monkeys with MMC-treated BLCL-HM induced Her-2-specific IFN-γ secretion after 10 days of culture. When the culture supernatants of splenocytes were assessed using ELISA, most restimulated splenocytes from group 2 and group 3 monkeys secreted considerable Her-2-specific IFN-γ (> 1000 units/ml); IFN-γ secretion after Her-2-specific restimulation was not detected in only one monkey (Monkey #9) in Group 3. However, no difference was observed in IFN-γ secretion between monkeys in group 2 and 3 (Figure 4). In contrast, restimulation with BLCL-HM or BLCL did not result in significant IFN-γ secretion by splenocytes in group 1 monkeys. These results suggest that our vaccine strategy elicited effective memory-type splenocytes that secreted IFN-γ when re-stimulated by Her-2-expressing BLCL.

Her-2-specific cytotoxic T lymphocyte activities of splenocytes were significantly increased by genetic immunization

Using an in vitro CTL assay, we next assessed the Her-2-specific cytolytic activities of splenocytes from immunized monkeys. After incubation of splenocytes with MMC-treated BLCL-HM, Her-2-specific lysis of BLCL-HM cells was measured by a standard Cr-51 release assay. Because long-term in vitro culture of splenocytes with BLCL-HM may induce primary CTL responses against BLCL as well as restimulation of pre-established Her-2-specific memory CD8+ T cells, we added only 1 unit/ml of IL-2 once on day 8 and then used the in vitro CTL assay to evaluate re-stimulated CTL activity on day 10. Splenocytes from three monkeys in group 3, which were primed with pHM-hGM-CSF and AdHM followed by pHM-hGM-CSF boosting, efficiently lysed BLCL-HM, but not BLCL (Figure 5A). Although splenocytes from two group 2 monkeys (Monkey #4 and #5) that had been primed with AdHM and boosted with pHM-hGM-CSF showed enhanced specific lysis of BLCL-HM, they could also lyse BLCL to the levels of BLCL-HM lysis after 10 days of in vitro stimulation with MMC-treated BLCL-HM. Thus, the splenocytes form monkeys in group 2 were further incubated with BLCL-HM for 14 days and supplemented with 1unit/ml of IL-2 on day 8 and day 10. After prolonged incubation of splenocytes from monkeys in group 2, Her-2-specific lysis of BLCL-HM was slightly greater than that of BLCL, especially in Monkeys #4 and #5 (Figure 5B). However, we could detect no Her-2-specific cytolytic activity of splenocytes in Monkey #6 even after prolonged incubation with BLCL-HM. In contrast, splenocytes from saline-treated monkeys in group 1 did not

lyse either BLCL or BLCL-HM (Figure 5A). These results show that genetic vaccines using AdHM and pHM-hGM-CSF efficiently elicited CTL responses after Her-2-specific in vitro restimulation.

Safety evaluation of genetic vaccines in cynomolgus monkeys

Our evaluation of the toxicities of genetic vaccines using our immunization schema revealed no statistically significant differences in weight loss, unconsumed feed, diarrhea, or death in treated monkeys (data not shown). We monitored immunized monkeys for 22 weeks for odd ophthalmological findings and abnormalities in hepatic and renal chemistry and serum biochemistry (Table 1). To investigate vaccination-related effects on organs, monkeys were sacrificed on week 22 and autopsies performed. A total of 41 organs were isolated, weighed, and examined grossly and histopathologically. Treatment-related abnormalities were not observed in any organs including liver and heart (data not shown). These findings lead us to conclude that our genetic vaccines efficiently elicited Her-2-specific immune responses without causing immunization-related adverse effects in non-human primates.

Discussion

Her-2/*neu* has been of keen interest as a target for cancer immunotherapy for decades. Indeed, passive immunotherapies using Her-2/*neu*-specific Ab or T cells have been partially successful in combating Her-2/*neu*-expressing cancer as a second-line therapy for chemotherapy and surgery [12-14]. Furthermore, several antigen-presenting cell-based vaccines loaded with Her-2/*neu* antigenic peptide or Her-2/*neu* peptide-based vaccine alone could induce Her-2/*neu*-specific immune responses in cancer patients [15,16]. However, few studies have dealt with the immunogenicity and safety of Her-2/*neu* genetic vaccines in cancer patients or even in nonhuman primates, despite the abundance of data in rodent models [4,17-19]. Genetic vaccines have been developed that rely on the expression of antigen inside of cells by naked plasmid DNA or viral vectors after gene delivery into the immunized; these vaccines can elicit both humoral and cellular immune responses against expressed antigen [20]. Although we previously used a DNA vaccine expressing a truncated form of Her-2/*neu* to generate successful anti-tumor immunity against Her-2/*neu*-expressing tumor cells in a mouse model by eliciting human Her-2/*neu*-specific humoral and cellular immune responses [4,6], this approach may not be equally suitable in overcoming self-tolerance and inducing effective immune responses in humans. Thus, to properly examine the efficacy of genetic vaccines prior to clinical trials, the immunogenicity and the safety of the vaccines needed to be tested in large animals such as non-human primates [21]. In the current study, we have evaluated the immunogenicity and the safety of genetic vaccine approaches in non-human primates, establishing the basis for the clinical use of the Her-2/*neu* genetic vaccines.

Genetic vaccines including plasmid DNA vaccines made it possible to elicit effective immune responses against many antigens [22]. It was shown that DNA vaccine against Prostate-specific antigen (PSA) induced PSA-specific Ab production and lympho-proliferative responses in immunized macaques; the stimulated T cells from PSA-immunized rhesus macaques produced high levels of Th1 cytokine IFN-γ, but not of IL-4, without any adverse effects [23]. Likewise, the *i.m.* administration of DNA vaccine against Carcinoembryonic antigen (CEA) was reported to produce comparable immune responses in immunized monkeys [24]. However, human clinical trials of CEA DNA vaccine elicited only low levels of lympho-proliferative responses and could not induce humoral immune responses to CEA after vaccination [8]. Further, DNA vaccine alone could not generate detectable levels of immune responses in melanoma patients [9].

To increase the limited immunogenicity of plasmid DNA vaccines, several investigators have used recombinant viruses as a boost for DNA vaccines [25]. Although adenoviruses are widely used as vectors in clinical gene therapy trials, their use was limited because they induced adenovirus-specific innate and adaptive immune responses [26] that could be detrimental to the generation of tumor antigen-specific immune responses [9]. To reduce these adenovirus-specific responses, we designed prior immunizations with plasmid DNA vaccine. Subsequent DNA vaccination can also boost antigen-specific memory responses induced by DNA and/or adenovirus immunization. Therefore, we combined plasmid DNA and adenovirus to maintain prolonged antigen-specific immune responses in immunized hosts. Further, intravenous injection of adenovirus vector has been reported to cause

liver toxicity, because most of the intravenously administered adenovirus vectors were taken up by Kupffer cells in the liver, causing hepatotoxicity associated with the production of proinflammatory cytokines such as IL-6, TNF-α, and RANTES [27]. However, in active immunotherapy, activated innate immunity may contribute to the generation of robust immune responses against delivered gene product. In the current non-human primate study, *i.m.* injection of DNA and human Her-2/*neu*-expressing adenoviral vector elicited Her-2-specific humoral and cellular immune responses without causing any detectable levels of acute toxicity, including liver and cardiac toxicities.

With our genetic vaccines, we could elicit high levels of Her-2-specific antibodies capable of lysing Her-2/*neu*-expressing tumor cells via ADCC. Further, some of these antibodies could compete with Herceptin® in the binding inhibition assay (Figure 1C), leading us to conjecture that our Her-2/*neu* genetic vaccines may generate Her-2-specific antibodies that share epitope specificity with Herceptin® even in clinical trials. Although DNA vaccine alone could not elicit Her-2-specific Ab responses within 4 weeks after initial immunization, we could detect rapid increases in Abs titers following immunization with AdHM. In contrast, Her-2-specific Ab production was evoked within a week after administration with AdHM alone and plateaued in four weeks. Because the Ab responses elicited by genetic vaccines persist for at least 18 weeks, we could not observe the effect of DNA boosting in our scheme.

Although pre-immunization with pHM-hGM-CSF followed by immunization with AdHM did not dramatically affect humoral immune responses, it did lead to striking differences in cellular immune

responses. Immunization with AdHM alone remarkably increased Her-2-specific helper T cell responses, including the proliferation of splenocytes after Her-2-specific restimulation and their secretion of IFN-γ. However, Her-2/*neu* non-specific proliferation was increased by the pHM-hGM-CSF immunization given before AdHM immunization in two of three monkeys in Group 3, perhaps because of the strong immunostimulatory effect of consecutive immunization with DNA and adenoviral vaccines during the priming. In contrast, distinct Her-2-specific CTL activities were observed mainly in splenocytes of monkeys in Group 3 that were given pHM-hGM-CSF together with AdHM, whereas priming with AdHM immunization alone increased Her-2-specific target lysis only slightly more than non-specific lyses of BLCL, which were a bit higher than those in Group 3. Because we tested the cellular immune responses using splenocytes harvested at the end time point of the study, we could not conclude which of the vaccination regimens most effectively generated anti-tumor immunity at the immune induction phase; the influence of MHC heterogeneities among monkeys on immune responses also needs further consideration. However, because Her-2 genetic vaccines elicited considerable levels of humoral immune responses in all monkeys to which they were administered, we can presume that various levels of Her-2-specific cellular immune responses may also be generated in immunized monkeys. Actually, in the case of Monkey #9, we observed no Her-2-specific CTL and IFN-γ secretion of splenocytes despite Her-2-specific lympho-proliferative responses and Ab production.

Mounting evidence suggests that CD8+ T cells responses, particularly CTL activity, play a critical role

in controlling tumor eradication in tumor-bearing mice models and cancer patients [28,29]. Several vaccine strategies using HLA-A2-binding Her-2/*neu* CTL epitope peptide were reported to be safe and effective in eliciting CTL responses even in Her-2/*neu*-expressing cancer patients [30,31]. However, it is difficult to directly measure CTL activity in non-human primates, because little information is available on MHC class I-binding epitope peptide. Thus, as an alternative, autologous B lymphoblastoid cell-lines (BLCL) transduced with retroviral vector–expressing antigen have been used as stimulator and target cells [32,33]. In the current study, we have evaluated the cytolytic activity of CD8+ T cells in the splenocytes of immunized monkeys, finding that stimulation with BLCL transduced with Her-2-expressing retroviral vector efficiently initiated CTL activity for the killing of Her-2/*neu*-expressing target cells.

Granulocyte macrophage colony–stimulating factor (GM-CSF), one of the most widely studied genetic adjuvants, has a good track record of enhancing anti-tumor immunity [34,35]. Although immunization with engineered tumor cells secreting low levels of GM-CSF generated measurable increases in antitumor effect, high-dose expression of GM-CSF instead impaired the immune responses by inducing the recruitment of myeloid-derived suppressor cells [36]. GM-CSF may also promote tumor cell transmigration through the endothelial barrier [37]. Further, high levels of GM-CSF secreted by cancer cells were shown to promote osteolytic bone metastasis [38]. Likewise, in clinical trials of GM-CSF-based vaccine, some patients with metastatic melanoma showed rapid disease progression [39]. However, because *i.m.* administration of pHM-hGM-CSF led to only low levels of local GM-CSF

secretion (Figure 1b) which only last for 14 days (data not shown) and because most GM-CSF-related adverse effects stemmed from high-dose secretion of GM-CSF, our strategy might not cause GM-CSF-derived adverse effects in clinical trials.

The primary safety concern of a clinical Her-2-based vaccine is the development cardiotoxicity which is the dominant clinical toxicity might be induced by Herceptin® administration after chemotherapy [40]. Although Herceptin-like antibodies were generated by our Her-2/neu vaccines, there was neither inflammatory process nor cardiac dysfunction in all of the immunized monkeys. It might be due to relatively low titers of the specific antibodies or due to the discrepancy in amino acid sequences between human and monkey. However, since we obtained data from the limited numbers of monkeys, further investigations should be made to verify the risk of anti-Her-2/neu-specific antibodies related cardiotoxicity by Her-2-based vaccines.

Although many of the genetic vaccines that have been tested to date were found to be safe and well tolerated, no good correlation has been established between positive lympho-proliferation and stable diseases [8,21,24]. To assess the immunogenicity of our vaccines in clinical trial, we tested the efficacy and the safety of these vaccines in non-human primates. Our genetic vaccines provided a clear advantage over passive Ab therapy; namely, they efficiently and simultaneously elicited humoral and cellular immune responses, including CTL activity as well as lympho-proliferation and IFN-γ secretion after Her-2-specific restimulation. Further, because some chemotherapies, especially gemcitabine or cyclophosphamide treatment, can attenuate the tumor-suppressive environment by

eliminating myeloid-derived suppressor cells [7] or tumor-induced regulatory T cells [41], in addition to directly killing tumor cells via their the intrinsic anti-neoplastic nature, genetic vaccines can be administered after chemotheraphy for the treatment of human cancer. In this regard, our vaccine might be used to develop both a therapeutic vaccine for reducing metastasis after chemotherapy and a prophylactic vaccine for people from genetically high-risk populations.

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Supplementary information is available at Gene Therapy's website

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Titles and legends to figures

Figure 1. Products of transgene were expressed in cells delivered with pHM-hGM-CSF or AdHM.

A. 293T cells were transfected with 2 μg pHM-hGM-CSF and harvested 2 days after transfection. The level of HM expression (line) was determined using FACS. B. The supernatants of 293T cells transfected with pHM-hGM-CSF were collected and filtered using 0.22-μm membrane filter. The concentration of hGM-CSF was measured by ELISA. C. CT26 cells were transduced with AdHM (MOI = 100) and harvested 2 days post-infection. The expression of HM (line) was analyzed by FACS. D. Immunoblot was also performed to examine the expression of HM in both 293T cells transfected with pHM-hGM-CSF and CT26 cells transduced with AdHM.

Figure 2. Genetic immunization of monkeys generated Her-2/neu-specific humoral immune responses.

Sera from immunized monkey were obtained weekly. A. The Her-2/*neu*-binding activities of antibodies in sera from immunized monkeys were measured using a SK-BR-3 assay. Titers were determined by a 2-fold increase in mean fluorescence intensity (MFI) of SK-BR-3 binding over that of the respective pre-immune sera. B. The Herceptin®-binding inhibition assay was conducted. Her2/ECD-coated plates were incubated with 6.25 ng/ml of biotin-labeled Herceptin® and diluted sera. The percentage of inhibition was calculated as [(6.25-converted Herceptin® concentration)/6.25]x100. C. The antibody-dependent killing of Her-2/*neu*-expressing cells was measured using sera of

immunized monkeys obtained at week 20 after immunization using human PBMC as effectors. Cr-51-labeled SK-BR-3 was incubated with diluted sera for 15 minutes before the addition of human PBMC. After 4 hours, Her-2/*neu*-specific lysis was measured by detecting Cr-51 release in the supernatant. As a positive control, ADCC of diluted Herceptin® (200ng/ml) was assessed.

Figure 3. Proliferation of splenocytes obtained from immunized monkeys after antigenic stimulation.

Splenocytes from groups of monkeys were co-cultured with the respective MMC-treated BLCL or BLCL-HM at the indicated effector:stimulator ratio for 96 hours, including a final 24-hour pulse with [^{3}H]thymidine. The cells were harvested and the incorporation of [^{3}H] thymidine measured. The value given represents the mean. Data are representative of at least two independent experiments.

Figure 4. Her-2/neu-specific IFN-γ secretion occurred during in vitro culture of splenocytes from immunized monkeys.

$5x10^6$ cells of splenocytes and $2.5x10^5$ cells of MMC-treated stimulator cells were co-incubated in 1ml of culture media for 10 days; 1U/ml of human IL-2 was added at day 8. The concentrations of simian IFN-γ in culture supernatants were measured by ELISA. The negative O.D. values were expressed as under the detection limit (<10 U/ml). The data shown are representative of two separate experiments.

Figure 5. Splenocytes obtained from immunized monkeys destroyed corresponding BLCL-HM in a Her-2/neu-specific manner.

A. Splenocytes from each immunized monkey were stimulated with MMC-treated BLCL-HM at a ration of 20:1 for 10 days. At day 8, 1U/ml of human IL-2 was added to splenocytes. Cr-51-labeled BLCL and BLCL-HM were used as targets. B. Splenocytes of monkeys in group 2 were cultured with MMC-treated BLCL-HM at a ratio of 20:1 for 14 days. At days 8, 10, and 12, 1U/ml of human IL-2 was added. Her-2/*neu*-specific lysis was measured using a 4-hour standard Cr-51-release assay. Each point represents the mean value. The data shown are representative of at least two separate experiments.

Figure 1

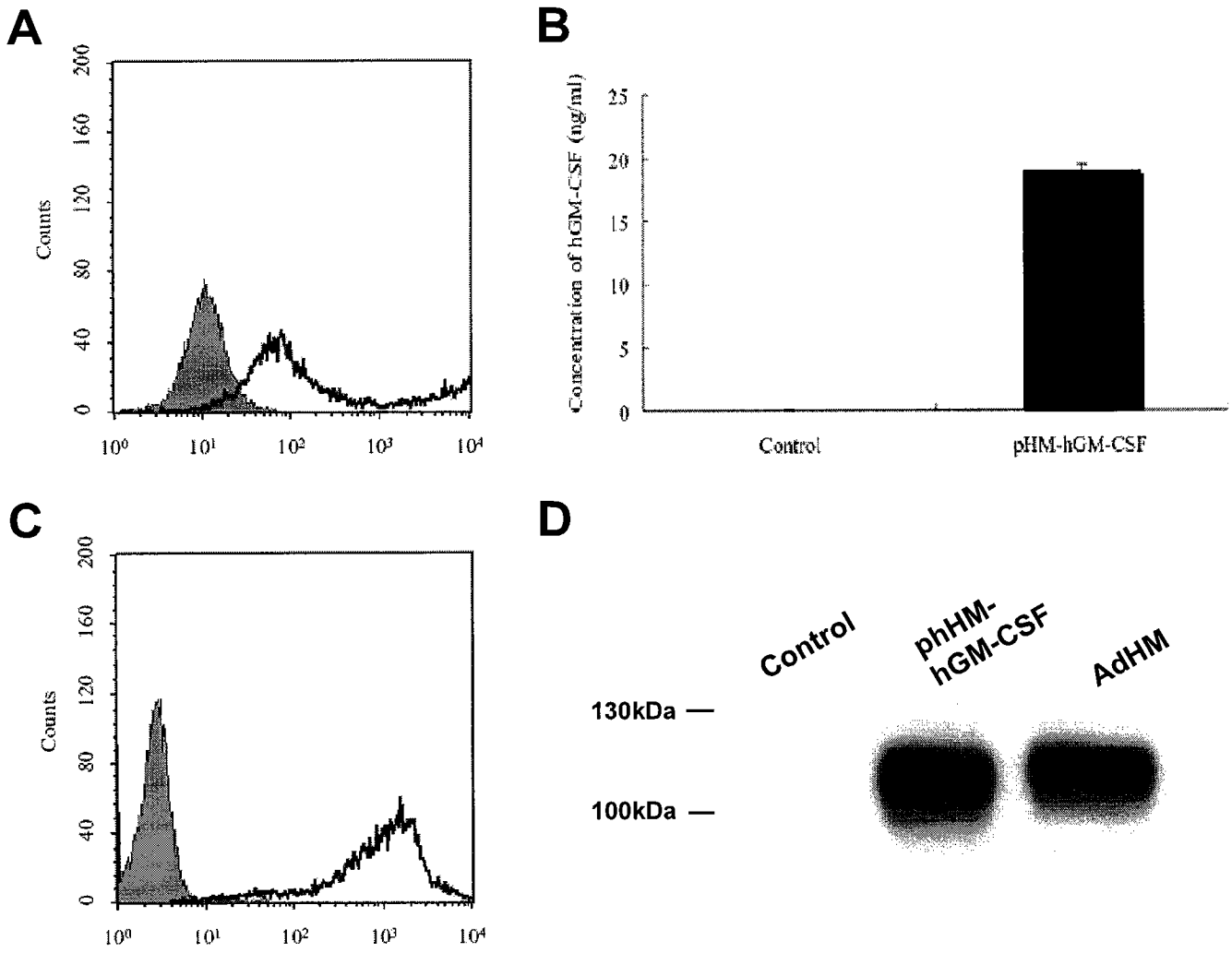
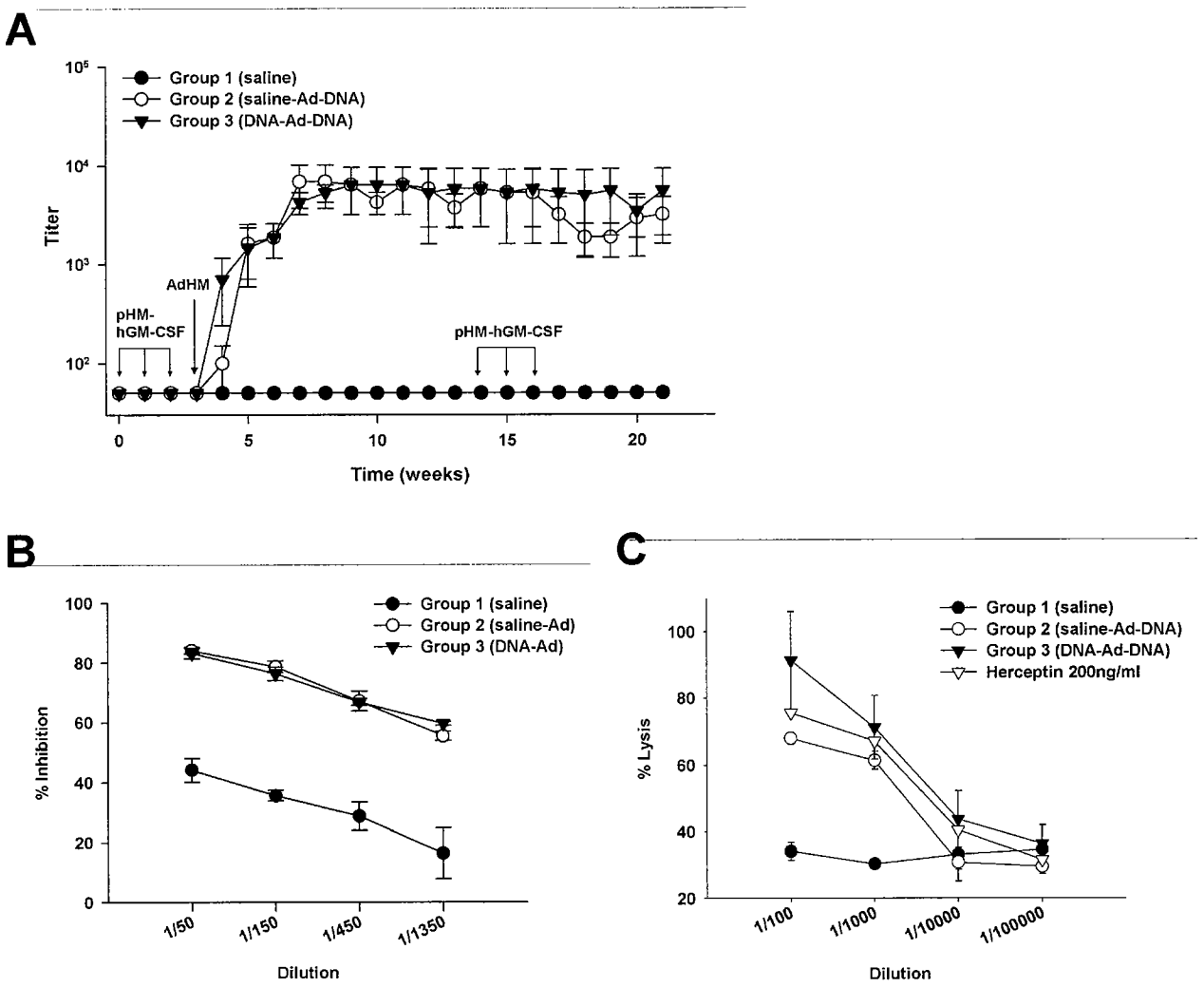

A
Counts
0
40
80
120
160
200
10^0
10^1
10^2
10^3
10^4
B
Concentration of hGM-CSF (ng/ml)
0
5
10
15
20
25
**
Control
pHM-hGM-CSF
C
Counts
0
40
80
120
160
200
10^0
10^1
10^2
10^3
10^4
D
Control
phHM-hGM-CSF
AdHM
130kDa —
100kDa —

Figure 2


A
Group 1 (saline)
Group 2 (saline-Ad-DNA)
Group 3 (DNA-Ad-DNA)
Titer
AdHM
pHM-hGM-CSF
pHM-hGM-CSF
Time (weeks)
B
Group 1 (saline)
Group 2 (saline-Ad)
Group 3 (DNA-Ad)
% Inhibition
Dilution
C
Group 1 (saline)
Group 2 (saline-Ad-DNA)
Group 3 (DNA-Ad-DNA)
Herceptin 200ng/ml
% Lysis
Dilution

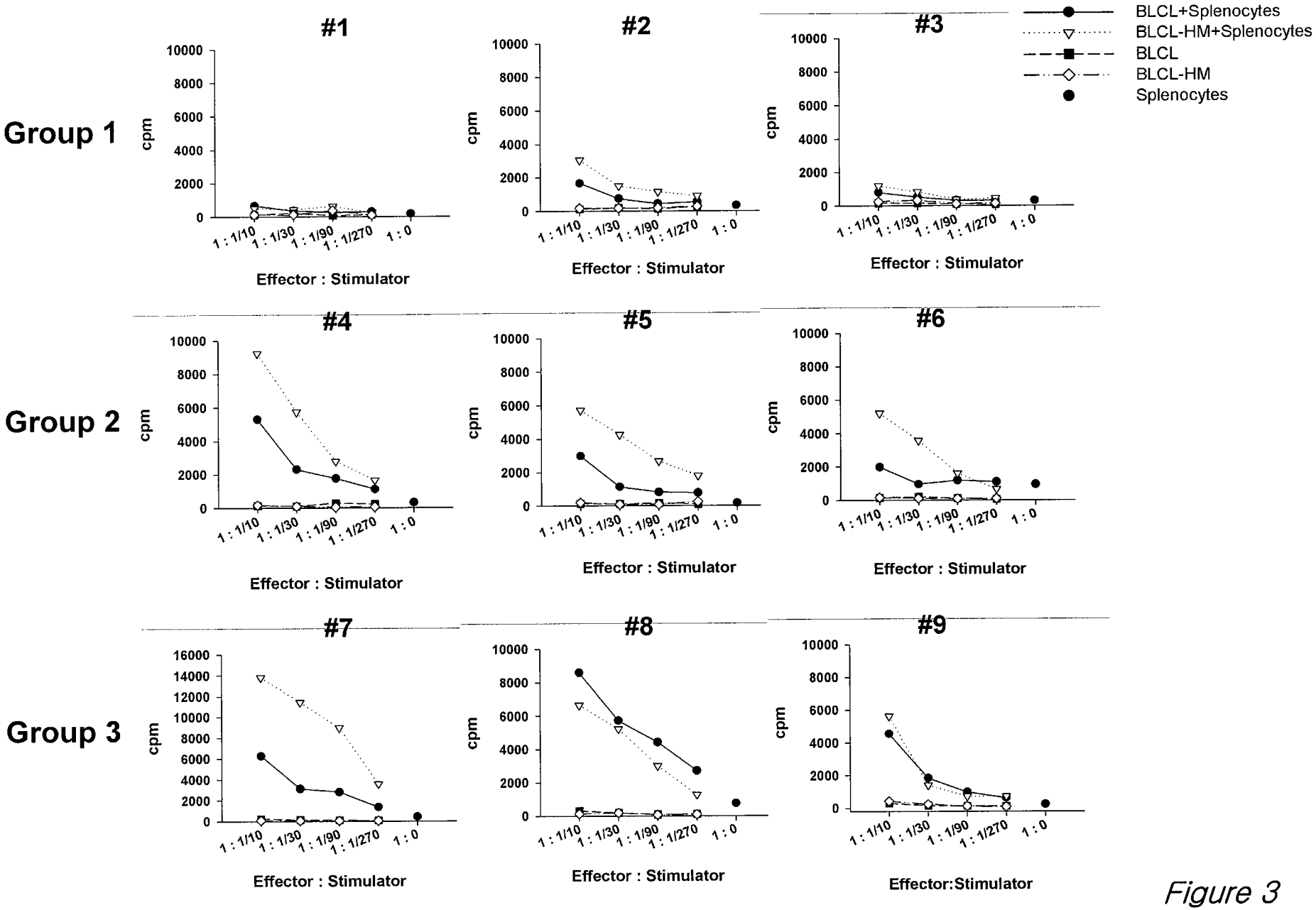
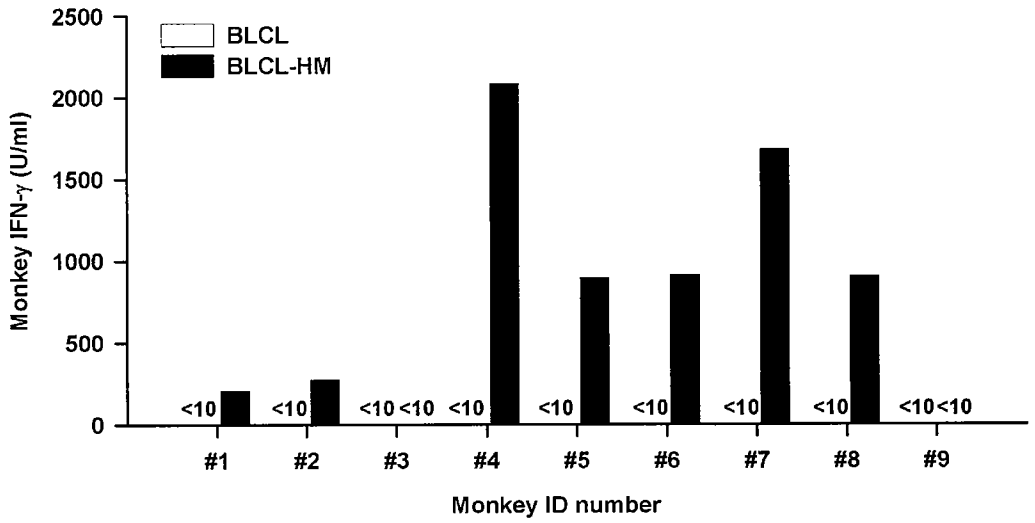

Group 1
Group 2
Group 3
#1
#2
#3
#4
#5
#6
#7
#8
#9
cpm
Effector : Stimulator
Effector:Stimulator
1 : 1/10
1 : 1/30
1 : 1/90
1 : 1/270
1 : 0
BLCL+Splenocytes
BLCL-HM+Splenocytes
BLCL
BLCL-HM
Splenocytes

Figure 3

Figure 4


2500
2000
1500
1000
500
0
Monkey IFN-γ (U/ml)
BLCL
BLCL-HM
<10
<10
<10 <10
<10
<10
<10
<10
<10
<10 <10
#1
#2
#3
#4
#5
#6
#7
#8
#9
Monkey ID number

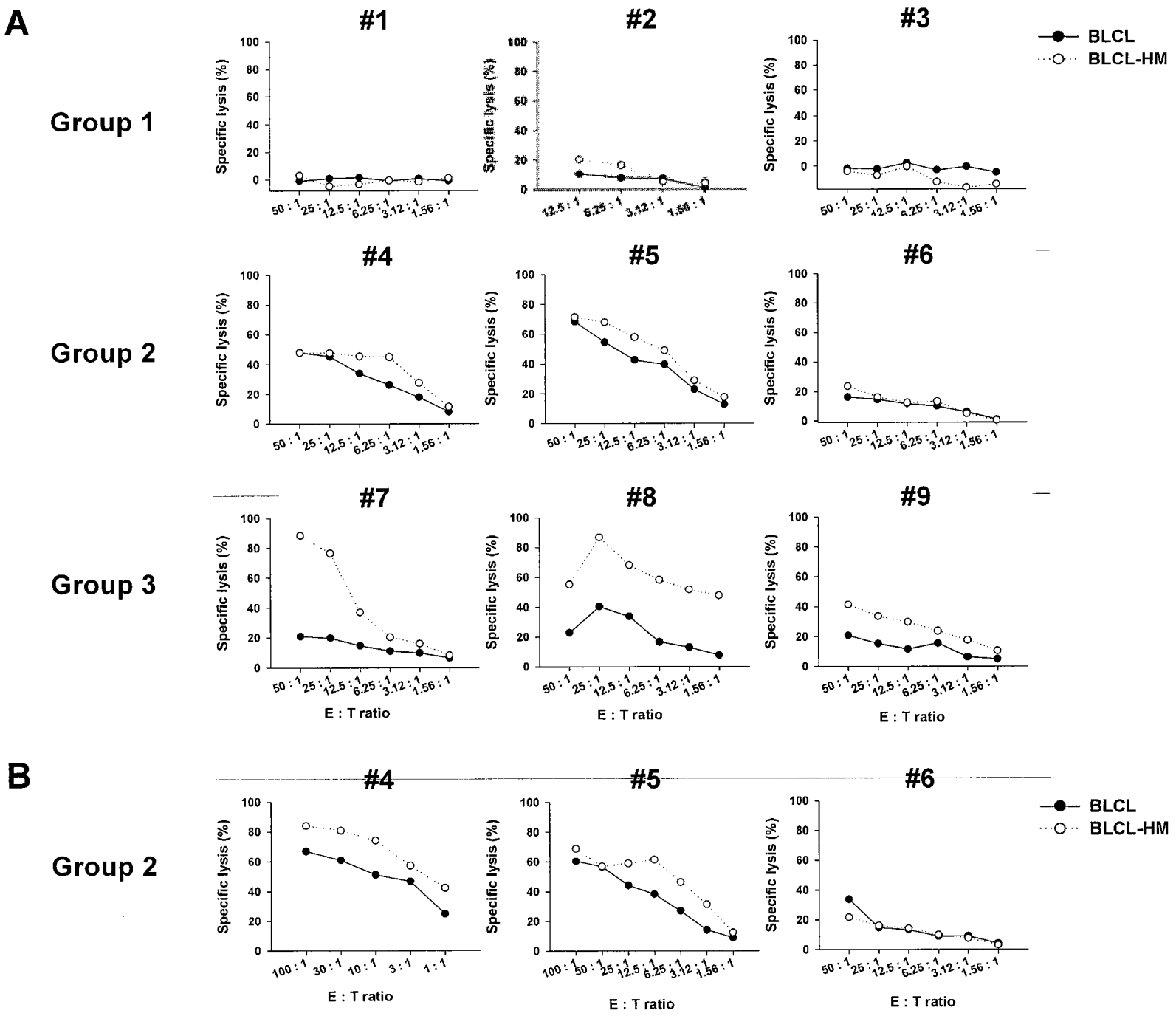

A
Group 1
Group 2
Group 3
#1
#2
#3
#4
#5
#6
#7
#8
#9
BLCL
BLCL-HM
Specific lysis (%)
E : T ratio
50 : 1
25 : 1
12.5 : 1
6.25 : 1
3.12 : 1
1.56 : 1
B
Group 2
#4
#5
#6
BLCL
BLCL-HM
Specific lysis (%)
100 : 1
30 : 1
10 : 1
3 : 1
1 : 1
E : T ratio

Figure 5

Talbe 1

A. Hematological values

	Group 1				Group 2				Group 3			
	Pretest	Day 35	Day 126	Day 154	Pretest	Day 35	Day 126	Day 154	Pretest	Day 35	Day 126	Day 154
WBC (x10^3/μℓ)	11.83 (±5.50)	11.18 (±3.48)	12.44 (±2.74)	9.72 (±2.67)	10.54 (±4.27)	13.55 (±4.01)	13.37 (±8.66)	11 (±7.11)	9.44 (±0.91)	12.69 (±3.19)	10.36 (±2.41)	7.84 (±2.08)
RBC (x10^6/μℓ)	5.05 (±0.18)	5.31 (±0.13)	5.35 (±0.1)	4.98 (±0.15)	5.15 (±0.18)	5.05 (±0.15)	5.06 (±0.34)	4.82 (±0.15)	5.53 (±0.39)	5.64 (±0.19)	5.62 (±0.24)	5.18 (±0.22)
HGB (g/dL)	12.3 (±0.35)	13.2 (±0.35)	13.6 (±0.26)	13 (±0.35)	12.7 (±0.72)	12.9 (±0.40)	13 (±1.05)	12.7 (±0.65)	12.8 (±1.25)	13.4 (±0.61)	13.5 (±0.76)	12.7 (±0.84)
HCT (%)	42.6 (±1.92)	42.9 (±2.76)	42.8 (±0.55)	39.5 (±0.60)	43.7 (±2.20)	41.1 (±1.62)	41 (±3.26)	38.9 (±1.27)	46.0 (±4.75)	44.0 (±1.97)	44.2 (±2.57)	39.9 (±1.98)
MCV (fL)	84.5 (±2.00)	80.9 (±3.27)	80.2 (±1.43)	79.2 (±1.21)	84.7 (±1.40)	82.2 (±1.59)	80.8 (±1.53)	80.6 (±0.68)	83.2 (±4.15)	77.9 (±1.66)	78.7 (±1.88)	77 (±2.25)
MCH (pg)	24.3 (±0.26)	24.8 (±0.25)	25.5 (±0.21)	26.2 (±0.15)	24.5 (±0.55)	25.5 (±0.17)	25.6 (±0.72)	26.3 (±0.84)	23.1 (±0.56)	23.7 (±0.32)	24.1 (±0.35)	24.6 (±0.70)
MCHC (g/dL)	28.8 (±0.40)	30.7 (±1.11)	31.8 (±0.46)	33 (±0.40)	29 (±0.15)	31 (±0.49)	31.6 (±0.40)	32.6 (±0.75)	27.8 (±1.30)	30.4 (±0.32)	30.7 (±0.40)	31.9 (±0.80)
PLT (x10^3/μℓ)	348 (±12.0)	356 (±13.2)	283 (± 79.4)	326 (±68.6)	380 (±60.7)	379 (±37.2)	379 (±63.1)	400 (±82.3)	343 (±50.3)	369 (±28.5)	339 (±77.7)	369 (±57.4)
CHCM (g/dL)	28.97 (±0.97)	31.3 (±1.57)	31.3 (±0.82)	32.07 (±0.71)	29.37 (±0.45)	31.07 (±0.84)	31.4 (±0.20)	32 (±0.78)	27.73 (±1.50)	30.2 (0.66)	29.6 (±0.17)	30.9 (±0.79)
CH	24.4 (±0.26)	25.2 (±0.40)	25 (±0.31)	25.3 (±0.21)	24.8 (±0.67)	25.5 (±0.76)	25.4 (±0.64)	25.8 (±0.81)	23.0 (±0.64)	23.5 (±0.55)	23.3 (±0.47)	23.7 (±0.56)
RET% (%)	2 (±0.47)	1.5 (±0.23)	1.6 (±0.15)	1.7 (±0.35)	1.7 (±0.35)	1.8 (±0.59)	2.1 (±0.64)	1.8 (±0.47)	2.3 (±0.31)	1.1 (±0.35)	1.6 (±0.17)	2 (±0.15)
NEU% (%)	39.8 (±10.76)	28.47 (±3.30)	32.63 (±7.81)	34.63 (±7.43)	46.27 (±4.34)	35.87 (±10.23)	46.13 (±14.01)	47.27 (±14.09)	46.33 (±7.76)	43.23 (±13.56)	46.87 (±5.22)	42.87 (±9.93)
LYM% (%)	55.8 (±9.97)	67.4 (±2.95)	62.5 (±8.59)	59.5 (±8.67)	49.3 (±5.35)	54.9 (±8.50)	48.7 (±12.45)	46.7 (±11.64)	48.5 (±6.19)	48.7 (±11.39)	47.2 (±4.16)	51.7 (±8.30)
EOS% (%)	1.3 (±0.53)	1 (±0.44)	1.2 (±0.85)	2.1 (±2.08)	0.8 (±0.45)	5.6 (±4.62)	0.7 (±0.31)	0.7 (±0.15)	1.5 (±0.55)	5.0 (±1.83)	1.7 (±0.60)	1.4 (±0.90)
MON% (%)	2 (±0.90)	2.2 (±0.91)	2.7 (±0.64)	3 (±1.27)	2.7 (±0.80)	2.3 (±0.75)	3.9 (±2.00)	4.5 (±2.37)	2.8 (±0.98)	1.9 (±0.36)	3.6 (±0.58)	3.5 (±0.96)
BAS% (%)	0.6 (±0.12)	0.4 (±0.21)	0.6 (±0.25)	0.5 (±0.38)	0.5 (±0.00)	0.6 (±0.10)	0.3 (±0.06)	0.3 (±0.15)	0.4 (±0.10)	0.5 (±0.21)	0.3 (±0.12)	0.2 (±0.12)
LUC% (%)	0.5 (±0.15)	0.5 (±0.05)	0.4 (±0.00)	0.3 (±0.06)	0.4 (±0.25)	0.9 (±0.46)	0.3 (±0.10)	0.4 (±0.23)	0.5 (±0.20)	0.7 (±0.23)	0.4 (±0.20)	0.4 (±0.10)

B. Serum biological values

	Group 1				Group 2				Group 3			
	Pretest	Day 35	Day 126	Day 154	Pretest	Day 35	Day 126	Day 154	Pretest	Day 35	Day 126	Day 154
GLU (mg/dL)	67.9 (±14.04)	79.6 (±16.49)	77.1 (±13.77)	74.7 (±5.74)	61.5 (±10.93)	80.7 (±29.89)	71.2 (±12.11)	78.5 (±9.39)	73.3 (±9.93)	71.3 (±7.36)	65.5 (±3.55)	69.6 (±7.5)
BUN (mg/dL)	15.3 (±2.48)	15.7 (±4.8)	17.7 (±3.27)	16.6 (±1.63)	20.4 (±4.69)	19.2 (±2.31)	20 (±2.41)	21 (±1.8)	14 (±2.19)	17 (±4.3)	20 (±4.91)	19 (±5.99)
CREA (mg/dL)	0.81 (±0.09)	0.85 (±0.06)	0.89 (±0.11)	0.95 (±0.16)	0.91 (±0.11)	0.9 (±0.19)	1.78 (±1.16)	0.99 (±0.14)	0.97 (±0.04)	0.92 (±0.05)	1 (±0.06)	0.97 (±0.09)
TP (g/dL)	7.14 (±0.46)	7.18 (±0.36)	7.4 (±0.36)	7.29 (±0.18)	7.3 (±0.29)	7.09 (±0.10)	7.12 (±0.46)	7.45 (±0.38)	7.11 (±0.31)	7.44 (±0.7)	7.33 (±0.29)	7.48 (±0.53)
ALB (g/dL)	4.2 (±0.22)	4.16 (±0.22)	4.28 (±0.19)	4.3 (±0.04)	4.27 (±0.13)	4.01 (±0.23)	4.18 (±0.17)	4.47 (±0.16)	4 (±0.24)	4.06 (±0.33)	4.1 (±0.16)	4.29 (±0.26)
A/G (ratio)	1.43 (±0.11)	1.38 (±0.11)	1.37 (±0.13)	1.44 (±0.06)	1.41 (±0.04)	1.3 (±0.13)	1.43 (±0.09)	1.51 (±0.08)	1.28 (±0.08)	1.2 (±0.03)	1.26 (±0.01)	1.35 (±0.04)
TCHO (mg/dL)	106.5 (±15.7)	109.9 (±8.36)	115.6 (±9.7)	123.4 (±17.44)	112.5 (±30.1)	117.3 (±21.45)	123.5 (±12.31)	135.9 (±14.76)	122.4 (±35.82)	125.6 (±27.25)	129.8 (±27.96)	143.3 (±38.06)
TG (mg/dL)	24.5 (±13.16)	27.6 (±7.05)	47.7 (±16.38)	27.2 (±9.18)	31.5 (±5.23)	32.1 (±16.76)	40.5 (±27.01)	29.9 (±9.3)	52.5 (±8.77)	40.3 (±13.46)	69.2 (±32.9)	42.3 (±14.97)
PL (mg/dL)	158 (±25.4)	175 (±19.4)	202 (±19.9)	177 (±16.6)	181 (±37.4)	184 (±21.5)	205 (±26.8)	202 (±30.6)	201 (±41.9)	206 (±7.9)	234 (±19.7)	216 (±19.5)
AST (IU/L)	33 (±5.78)	37.4 (±6.82)	36 (±5.18)	34.1 (±6.01)	51.3 (±11.59)	41.4 (±7.28)	42.2 (±7.33)	36.3 (±2.9)	29.2 (±3.77)	32 (±3.96)	35.3 (±4.39)	27.8 (±2.18)
ALT (IU/L)	39.3 (±15.37)	37.5 (±16.57)	33.1 (±17.08)	30.2 (±12.54)	41.4 (±13.47)	36.3 (±6.19)	36.7 (±9.77)	39.6 (±14.06)	40.7 (±19.04)	29.9 (±9.42)	37.4 (±6.73)	39.6 (±6.99)
TBIL (mg/dL)	0.23 (±0.02)	0.2 (±0.04)	0.2 (±0.02)	0.211 (±0.08)	0.25 (±0.09)	0.18 (±0.02)	0.224 (±0.05)	0.192 (±0.03)	0.18 (±0.04)	0.15 (±0.03)	0.213 (±0.1)	0.132 (±0.03)
ALP (IU/L)	785.1 (±102.4)	771.4 (±132.9)	679.8 (±102.6)	671.7 (±136.97)	553.3 (±88.62)	400.1 (±24.25)	461.7 (±71.27)	462.3 (±94.05)	374.6 (±87.86)	380.6 (±109.9)	278.1 (±154.71)	316.1 (±82.76)
CK (IU/L)	241 (±133.7)	285 (±86.3)	306 (±192.7)	525 (±542.9)	458 (±275.3)	256 (±44.4)	227 (±61.1)	177 (±23.6)	285 (±160.4)	580 (±375.3)	286 (±131.2)	153 (±38.4)
Ca (mg/dL)	9.77 (±0.35)	9.73 (±0.34)	10.24 (±0.16)	10.10 (±0.411)	10.07 (±0.25)	9.55 (±0.55)	10 (±0.66)	10.3 (±0.47)	9.80 (±0.3)	9.68 (±0.65)	10.32 (±0.59)	10.22 (±0.6)
IP (mg/dL)	5.03 (±0.55)	4.93 (±0.44)	3.94 (±0.4)	4.68 (±0.56)	4.41 (±0.73)	4.39 (±0.65)	3.83 (±0.99)	4.47 (±0.26)	4.15 (±1.04)	3.69 (±0.4)	2.86 (±0.67)	4.02 (±0.96)
Na (mmol/L)	145 (±1.2)	145 (±1.0)	144 (±1.5)	148 (±1.5)	143 (±1.2)	142 (±1.2)	143 (±1.5)	149 (±2.1)	146 (±0.6)	145 (±0.6)	147 (±0.6)	151 (±1.0)
K (mmol/L)	3.8 (±0.23)	3.64 (±0.61)	3.81 (±0.35)	4.36 (±0.57)	4.06 (±0.12)	4.1 (±0.27)	4.09 (±0.29)	4.54 (±0.59)	4.48 (±0.2)	4.25 (±0.05)	4.45 (±0.24)	4.67 (±0.24)
Cl (mmol/L)	107 (±2.3)	107 (±2.1)	109 (±2.3)	111 (±3.2)	106 (±0.6)	106 (±2.1)	109 (±1.5)	113 (±0.6)	110 (±2.0)	110 (±1.7)	112 (±2.5)	114 (±2.5)

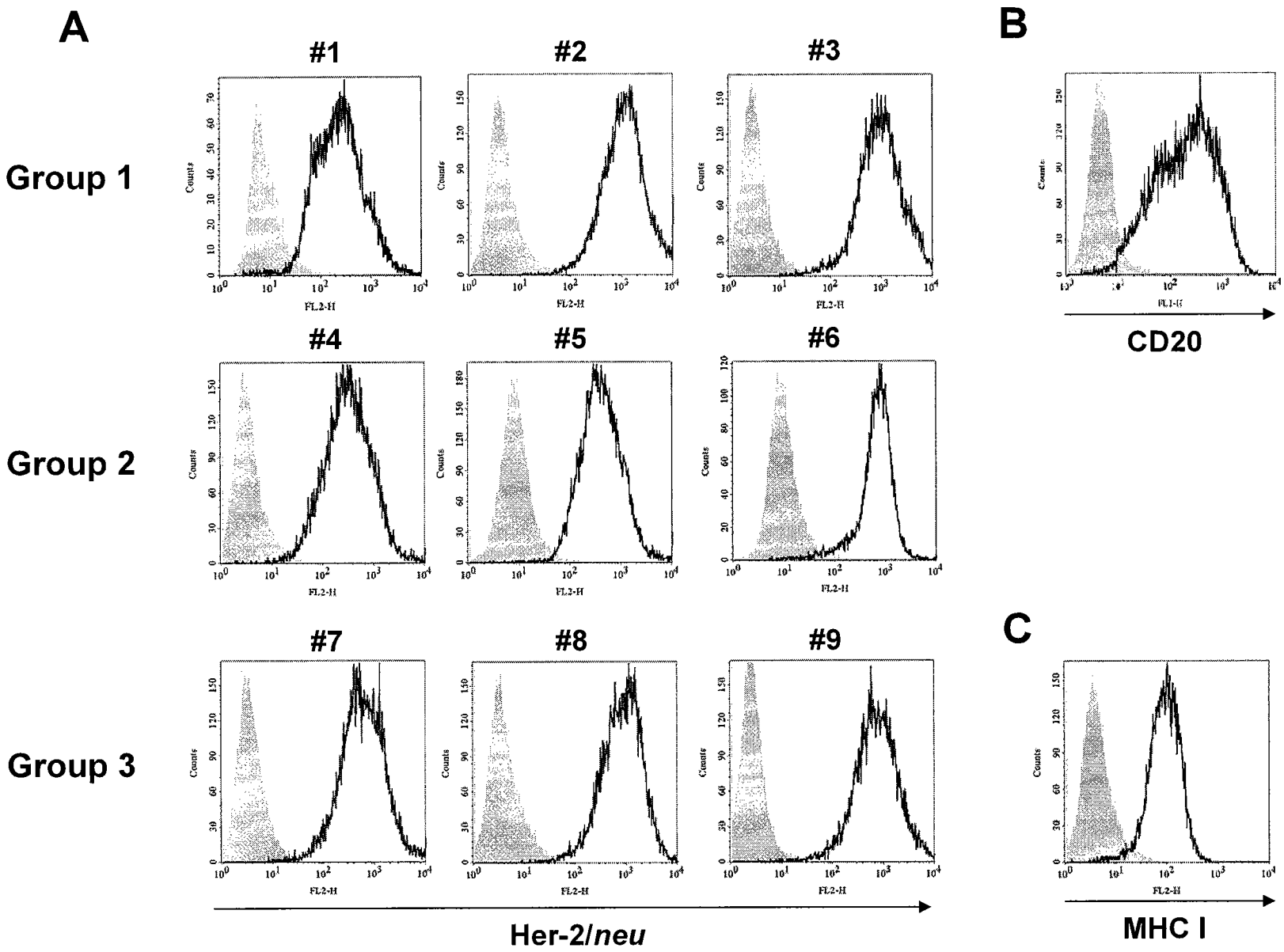
Supplementary Figure 1 Groups and experimental schema of simian model

Group	No. of Animals	Animal ID	Dose
1	3 females	1-3	[1]Sal: 2ml/head
2	3 females	4-6	[2]DNA: 4mg/2ml/head, [3]Ad: 1×10^{10}pfu/1ml/head
3	3 females	7-9	DNA: 4mg/2ml/head, Ad: 1×10^{10}pfu/1ml/head

Group	Weeks									
	0	1	2	3	4-13	14	15	16	17-21	22
1	Sal	Sal	Sal	Sal	-	Sal	Sal	Sal	-	Autopsy
2	Sal	Sal	Sal	Ad	-	DNA	DNA	DNA	-	
3	DNA	DNA	DNA	Ad	-	DNA	DNA	DNA	-	

[1]Sal; saline, [2]DNA; pHM-hGM-CSF, [3]Ad; AdHM

Supplementary Figure 2



Supplementary Figure 1. Confirmation of Her-2/neu expression on autologous B lymphoblastoid cells (BLCL) transduced with retroviral vector expressing Her-2/neu.

A. Autologous BLCL and BLCL-HM were stained with PE-conjugated anti-Her-2/*neu* antibody. Binding of anti-Her-2/*neu* Ab was analyzed by flow cytometry. BLCL is shown as a filled area, and BLCL-HM is shown as a solid line. B. BLCL #1 was stained with FITC-conjugated anti-human CD20 antibody. CD20 expression is shown as a solid line. Binding of the control antibody is shown as a filled area. C. BLCL #1 was stained with PE-conjugated anti-human MHC I antibody. The level of MHC I is shown as a solid line. Binding of the control antibody is shown as a filled area. Data are representative of at least three separate experiments.